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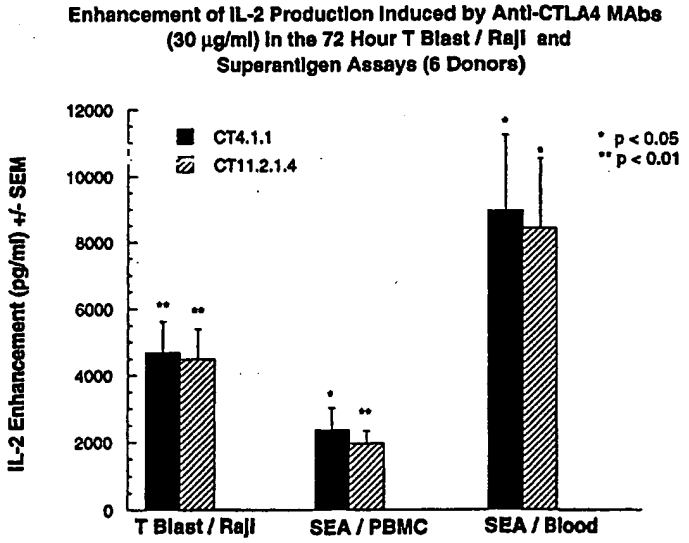

(57) Abstract

In accordance with the present invention, there are provided fully human monoclonal antibodies against human cytotoxic T–lymphocyte antigen 4 (CTLA–4). Nucleotide sequences encoding and amino acid sequences comprising heavy and light chain immunoglobulin molecules, particularly contiguous heavy and light chain sequences spanning the complementarity determining regions (CDRs), specifically from within FR1 and/or CDR1 through CDR3 and/or within FR4, are provided. Further provided are antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein.

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HUMAN MONOCLONAL ANTIBODIES TO CTLA-4

BACKGROUND OF THE INVENTION

1. *Cross-Reference to Related Applications*

The present application claims priority to U.S. Provisional Patent Application, Serial No. 60/113,647, filed December 23, 1998, the disclosure of which is hereby incorporated in its entirety herein.

2. *Summary of the Invention*

In accordance with the present invention, there are provided fully human monoclonal antibodies against human cytotoxic T-lymphocyte antigen 4 (CTLA-4). Nucleotide sequences encoding and amino acid sequences comprising heavy and light chain immunoglobulin molecules, particularly contiguous heavy and light chain sequences spanning the complementarity determining regions (CDRs), specifically from within FR1 and/or CDR1 through CDR3 and/or within FR4, are provided. Further provided are antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein.

3. *Background of the Technology*

Regulation of immune response in patients would provide a desirable treatment of many human diseases that could lead to a specificity of action that is rarely found through the use of conventional drugs. Both up-regulation and down-regulation of responses of the immune system would be possible. The roles of T cells and B cells have been extensively studied and characterized in connection with the regulation of immune response. From these studies, the role of T cells appear, in many cases, to be particularly important in disease prevention and treatment.

T cells possess very complex systems for controlling their interactions. Interactions between T cells utilize numerous receptors and soluble factors for the process. Thus, what effect any particular signal may have on the immune response generally varies and depends on the particular factors, receptors and counter-receptors that are involved in the pathway. The pathways for down-regulating responses are as important as those required for activation. Thymic education leading to T-cell tolerance is one mechanism for preventing an immune response to a particular antigen. Other mechanisms, such as secretion of suppressive cytokines, are also known.

Activation of T cells requires not only stimulation through the antigen receptor (T cell receptor (TCR)), but additional signaling through co-stimulatory surface molecules such as CD28. The ligands for CD28 are the B7-1 (CD80) and B7-2 (CD86) proteins, which are expressed on antigen-presenting cells such as dendritic cells, activated B-cells or monocytes that interact with T-cell CD28 or CTLA-4 to deliver a costimulatory signal. The role of costimulatory signaling was studied in experimental allergic encephalomyelitis (EAE) by Perrin et al. *Immunol Res* **14**:189-99 (1995). EAE is an autoimmune disorder, induced by Th1 cells directed against myelin antigens that provides an *in vivo* model for studying the role of B7-mediated costimulation in the induction of a pathological immune response. Using a soluble fusion protein ligand for the B7 receptors, as well as monoclonal antibodies specific for either CD80 or CD86, Perrin et al. demonstrated that B7 costimulation plays a prominent role in determining clinical disease outcome in EAE.

The interaction between B7 and CD28 is one of several co-stimulatory signaling pathways that appear to be sufficient to trigger the maturation and proliferation of antigen specific T-cells. Lack of co-stimulation, and the concomitant inadequacy of IL-2 production, prevent subsequent proliferation of the T cell and induce a state of non-reactivity termed "anergy". A variety of viruses and tumors may block T cell activation and proliferation, leading to insufficient activity or non-reactivity of the host's immune system to the infected or transformed cells. Among a number of possible T-cell disturbances,

anergy may be at least partly responsible for the failure of the host to clear the pathogenic or tumorgenic cells.

The use of the B7 protein to mediate anti-tumor immunity has been described in Chen et al. *Cell* 71:1093-1102 (1992) and Townsend and Allison *Science* 259:368 (1993). Schwartz *Cell* 71:1065 (1992) reviews the role of CD28, CTLA-4, and B7 in IL-2 production and immunotherapy. Harding et al. *Nature* 356:607-609 (1994) demonstrates that CD28 mediated signaling co-stimulates murine T cells and prevents the induction of anergy in T cell clones. *See also* U.S. Patent Nos. 5,434,131, 5,770,197, and 5,773,253, and International Patent Application Nos. WO 93/00431, WO 95/01994, WO 95/03408, WO 95/24217, and WO 95/33770.

From the foregoing, it was clear that T-cells required two types of signals from the antigen presenting cell (APC) for activation and subsequent differentiation to effector function. First, there is an antigen specific signal generated by interactions between the TCR on the T-cell and MHC molecules presenting peptides on the APC. Second, there is an antigen-independent signal that is mediated by the interaction of CD28 with members of the B7 family (B7-1 (CD80) or B7-2 (CD86)). Exactly where CTLA-4 fit into the milieu of immune responsiveness was initially evasive. Murine CTLA-4 was first identified and cloned by Brunet et al. *Nature* 328:267-270 (1987), as part of a quest for molecules that are preferentially expressed on cytotoxic T lymphocytes. Human CTLA-4 was identified and cloned shortly thereafter by Dariavach et al. *Eur. J. Immunol.* 18:1901-1905 (1988). The murine and human CTLA-4 molecules possess approximately 76% overall sequence homology and approach complete sequence identity in their cytoplasmic domains (Dariavach et al. *Eur. J. Immunol.* 18:1901-1905 (1988)). CTLA-4 is a member of the immunoglobulin (Ig) superfamily of proteins. The Ig superfamily is a group of proteins that share key structural features of either a variable (V) or constant (C) domain of Ig molecules. Members of the Ig superfamily include, but are not limited to, the immunoglobulins themselves, major histocompatibility complex (MHC) class molecules (i.e., MHC class I and II), and TCR molecules.

In 1991, Linsley et al. *J. Exp. Med.* **174**:561-569 (1991), proposed that CTLA-4 was a second receptor for B7. Similarly, Harper et al. *J Immunol* **147**:1037-44 (1991) demonstrated that the CTLA-4 and CD28 molecules are closely related in both mouse and human as to sequence, message expression, gene structure, and chromosomal location. *See also* Balzano et al. *Int J Cancer Suppl* 7:28-32 (1992). Further evidence of this role arose through functional studies. For example, Lenschow et al. *Science* **257**:789-792 (1992) demonstrated that CTLA-4-Ig induced long term survival of pancreatic islet grafts. Freeman et al. *Science* **262**:907-909 (1993) examined the role of CTLA-4 in B7 deficient mice. Examination of the ligands for CTLA-4 are described in Lenschow et al. *P.N.A.S.* 90:11054-11058 (1993). Linsley et al. *Science* **257**:792-795 (1992) describes immunosuppression *in vivo* by a soluble form of CTLA-4. Linsley et al. *J Exp Med* **176**:1595-604 (1992) prepared antibodies that bound CTLA-4 and that were not cross-reactive with CD28 and concluded that CTLA-4 is coexpressed with CD28 on activated T lymphocytes and cooperatively regulates T cell adhesion and activation by B7. Kuchroo et al. *Cell* **80**:707-18 (1995) demonstrated that the B7-1 and B7-2 costimulatory molecules differentially activated the Th1/Th2 developmental pathways. Yi-qun et al. *Int Immunol* **8**:37-44 (1996) demonstrated that there are differential requirements for co-stimulatory signals from B7 family members by resting versus recently activated memory T cells towards soluble recall antigens. *See also* de Boer et al. *Eur J Immunol* **23**:3120-5 (1993).

Several groups proposed alternative or distinct receptor/ligand interactions for CTLA-4 as compared to CD28 and even proposed a third B-7 complex that was recognized by a BB1 antibody. *See, for example,* Hathcock et al. *Science* **262**:905-7 (1993), Freeman et al. *Science* **262**:907-9 (1993), Freeman et al. *J Exp Med* **178**:2185-92 (1993), Lenschow et al. *Proc Natl Acad Sci U S A* **90**:11054-8 (1993), Razi-Wolf et al. *Proc Natl Acad Sci U S A* **90**:11182-6 (1993), and Boussiotis et al. *Proc Natl Acad Sci U S A* **90**:11059-63 (1993). *But, see,* Freeman et al. *J Immunol* **161**:2708-15 (1998) who discuss finding that BB1 antibody binds a molecule that is identical to the cell surface form of CD74 and, therefore, the BB1 mAb binds to a protein distinct from B7-

1, and this epitope is also present on the B7-1 protein. Thus, this observation required the field to reconsider studies using BB1 mAb in the analysis of CD80 expression and function.

Beginning in 1993 and culminating in 1995, investigators began to further delineate the role of CTLA-4 in T-cell stimulation. First, through the use of monoclonal antibodies against CTLA-4, Walunas et al. *Immunity* 1:405-13 (1994) provided evidence that CTLA-4 can function as a negative regulator of T cell activation. Thereafter, Waterhouse et al. *Science* 270:985-988 (1995) demonstrated that mice deficient for CTLA-4 accumulated T cell blasts with up-regulated activation markers in their lymph nodes and spleens. The blast cells also infiltrated liver, heart, lung, and pancreas tissue, and amounts of serum immunoglobulin were elevated and their T cells proliferated spontaneously and strongly when stimulated through the T cell receptor, however, they were sensitive to cell death induced by cross-linking of the Fas receptor and by gamma irradiation. Waterhouse et al. concluded that CTLA-4 acts as a negative regulator of T cell activation and is vital for the control of lymphocyte homeostasis. In a comment in the same issue, Allison and Krummel *Science* 270:932-933 (1995), discussed the work of Waterhouse et al. as demonstrative that CTLA-4 acts to down regulate T-cell responsiveness or has an inhibitory signaling role in T-cell activation and development. Tivol et al. *Immunity* 3:541-7 (1995) also generated CTLA-4-deficient mice and demonstrated that such mice rapidly develop lymphoproliferative disease with multiorgan lymphocytic infiltration and tissue destruction, with particularly severe myocarditis and pancreatitis. They concluded that CTLA-4 plays a key role in down-regulating T cell activation and maintaining immunologic homeostasis. Also, Krummel and Allison *J Exp Med* 182:459-65 (1995) further clarified that CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. They generated an antibody to CTLA-4 and investigated the effects of its binding to CTLA-4 in a system using highly purified T cells. In their report, they showed that the presence of low levels of B7-2 on freshly explanted T cells can partially inhibit T cell proliferation, and this inhibition was mediated by interactions with CTLA-4. Cross-linking of CTLA-4 together with the TCR and CD28 strongly

inhibits proliferation and IL-2 secretion by T cells. Finally, the results showed that CD28 and CTLA-4 deliver opposing signals that appear to be integrated by the T cell in determining the response to antigen. Thus, they concluded that the outcome of T cell antigen receptor stimulation is regulated by CD28 costimulatory signals, as well as inhibitory signals derived from CTLA-4. *See also* Krummel et al. *Int Immunol* 8:519-23 (1996) and U.S. Patent No. 5,811,097 and International Patent Application No. WO 97/20574.

A variety of additional experiments have been conducted further elucidating the above function of CTLA-4. For example, Walunas et al. *J Exp Med* **183**:2541-50 (1996), through the use of anti-CTLA-4 antibodies, suggested that CTLA-4 signaling does not regulate cell survival or responsiveness to IL-2, but does inhibit CD28-dependent IL-2 production. Also, Perrin et al. *J Immunol* **157**:1333-6 (1996), demonstrated that anti-CTLA-4 antibodies in experimental allergic encephalomyelitis (EAE), exacerbated the disease and enhanced mortality. Disease exacerbation was associated with enhanced production of the encephalitogenic cytokines TNF-alpha, IFN-gamma and IL-2. Thus, they concluded that CTLA-4 regulates the intensity of the autoimmune response in EAE, attenuating inflammatory cytokine production and clinical disease manifestations. *See also* Hurwitz et al. *J Neuroimmunol* **73**:57-62 (1997) and Cepero et al. *J Exp Med* **188**:199-204 (1998) (an anti-CTLA-4 hairpin ribozyme that specifically abrogates CTLA-4 expression after gene transfer into a murine T-cell model).

In addition, Blair et al. *J Immunol* **160**:12-5 (1998) assessed the functional effects of a panel of CTLA-4 monoclonal antibodies (mAbs) on resting human CD4+ T cells. Their results demonstrated that some CTLA-4 mAbs could inhibit proliferative responses of resting CD4+ cells and cell cycle transition from G0 to G1. The inhibitory effects of CTLA-4 were evident within 4 h, at a time when cell surface CTLA-4 expression remained undetectable. Other CTLA-4 mAbs, however, had no detectable inhibitory effects, indicating that binding of mAbs to CTLA-4 alone was not sufficient to mediate down-regulation of T cell responses. Interestingly, while IL-2 production was shut off, inhibitory anti-CTLA-4 mAbs permitted induction and expression of the cell

survival gene bcl-X(L). Consistent with this observation, cells remained viable and apoptosis was not detected after CTLA-4 ligation.

In connection with anergy, Perez et al. *Immunity* 6:411-7 (1997) demonstrated that the induction of T cell anergy was prevented by blocking CTLA-4 and concluded that the outcome of antigen recognition by T cells is determined by the interaction of CD28 or CTLA-4 on the T cells with B7 molecules. Also, Van Parijs et al. *J Exp Med* **186**:1119-28 (1997) examined the role of interleukin 12 and costimulators in T cell anergy *in vivo* and found that through inhibiting CTLA-4 engagement during anergy induction, T cell proliferation was blocked, and full Th1 differentiation was not promoted. However, T cells exposed to tolerogenic antigen in the presence of both IL-12 and anti-CTLA-4 antibody were not anergized, and behaved identically to T cells which have encountered immunogenic antigen. These results suggested that two processes contribute to the induction of anergy *in vivo*: CTLA-4 engagement, which leads to a block in the ability of T cells to proliferate, and the absence of a prototypic inflammatory cytokine, IL-12, which prevents the differentiation of T cells into Th1 effector cells. The combination of IL-12 and anti-CTLA-4 antibody was sufficient to convert a normally tolerogenic stimulus to an immunogenic one.

In connection with infections, McCoy et al. *J Exp Med* **186**:183-7 (1997) demonstrated that anti-CTLA-4 antibodies greatly enhanced and accelerated the T cell immune response to *Nippostrongylus brasiliensis*, resulting in a profound reduction in adult worm numbers and early termination of parasite egg production. *See also* Murphy et al. *J. Immunol.* 161:4153-4160 (1998) (*Leishmania donovani*).

In connection with cancer, Kwon et al. *PNAS USA* **94**:8099-103 (1997) established a syngeneic murine prostate cancer model and examined two distinct manipulations intended to elicit an antiprostate cancer response through enhanced T cell costimulation: (i) provision of direct costimulation by prostate cancer cells transduced to express the B7.1 ligand and (ii) *in vivo* antibody-mediated blockade of T cell CTLA-4, which prevents T cell down-regulation. It was demonstrated that *in vivo* antibody-mediated blockade of CTLA-4

enhanced antiprostate cancer immune responses. Also, Yang et al. *Cancer Res* 57:4036-41 (1997) investigated whether the blockade of the CTLA-4 function leads to enhancement of antitumor T cell responses at various stages of tumor growth. Based on *in vitro* and *in vivo* results they found that CTLA-4 blockade in tumor-bearing individuals enhanced the capacity to generate antitumor T-cell responses, but the expression of such an enhancing effect was restricted to early stages of tumor growth in their model. Further, Hurwitz et al. *Proc Natl Acad Sci U S A* 95:10067-71 (1998) investigated the generation of a T cell-mediated antitumor response depends on T cell receptor engagement by major histocompatibility complex/antigen as well as CD28 ligation by B7. Certain tumors, such as the SM1 mammary carcinoma, were refractory to anti-CTLA-4 immunotherapy. Thus, through use of a combination of CTLA-4 blockade and a vaccine consisting of granulocyte-macrophage colony-stimulating factor-expressing SM1 cells, regression of parental SM1 tumors was observed, despite the ineffectiveness of either treatment alone. This combination therapy resulted in long-lasting immunity to SM1 and depended on both CD4(+) and CD8(+) T cells. The findings suggested that CTLA-4 blockade acts at the level of a host-derived antigen-presenting cell.

In connection with diabetes, Luhder et al. *J Exp Med* 187:427-32 (1998) injected an anti-CTLA-4 mAb into a TCR transgenic mouse model of diabetes at different stages of disease. They found that engagement of CTLA-4 at the time when potentially diabetogenic T cells are first activated is a pivotal event; if engagement is permitted, invasion of the islets occurs, but remains quite innocuous for months. If not, insulitis is much more aggressive, and diabetes quickly ensues.

In connection with vaccine immunization, Horspool et al. *J Immunol* 160:2706-14 (1998) found that intact anti-CTLA-4 mAb but not Fab fragments suppressed the primary humoral response to pCIA/beta gal without affecting recall responses, indicating CTLA-4 activation inhibited Ab production but not T cell priming. Blockade of the ligands for CD28 and CTLA-4, CD80 (B7-1) and CD86 (B7-2), revealed distinct and nonoverlapping function. Blockade of CD80 at initial immunization completely abrogated primary and secondary Ab

responses, whereas blockade of CD86 suppressed primary but not secondary responses. Simultaneous blockade of CD80 + CD86 was less effective at suppressing Ab responses than either alone. Enhancement of costimulation via coinjection of B7-expressing plasmids augmented CTL responses but not Ab responses, and without evidence of Th1 to Th2 skewing. These findings suggest complex and distinct roles for CD28, CTLA-4, CD80, and CD86 in T cell costimulation following nucleic acid vaccination.

In connection with allograft rejection, Markees et al. *J Clin Invest* **101**:2446-55 (1998) found in a mouse model of skin allograft rejection that acceptance initially depended on the presence of IFN-gamma, CTLA-4, and CD4(+) T cells. Addition of anti-CTLA-4 or anti-IFN-gamma mAb to the protocol was associated with prompt graft rejection, whereas anti-IL-4 mAb had no effect.

In connection with the role of CTLA-4 in relation to CD28, Fallarino et al. *J Exp Med* **188**:205-10 (1998) generated TCR transgenic/recombinase activating gene 2-deficient/CD28-wild-type or CD28-deficient mice which were immunized with an antigen-expressing tumor. Primed T cells from both types of mice produced cytokines and proliferated in response to stimulator cells lacking B7 expression. However, whereas the response of CD28+/+ T cells was augmented by costimulation with B7-1, the response of the CD28-/- T cells was strongly inhibited. This inhibition was reversed by monoclonal antibody against B7-1 or CTLA-4. Thus, CTLA-4 can potently inhibit T cell activation in the absence of CD28, indicating that antagonism of a TCR-mediated signal is sufficient to explain the inhibitory effect of CTLA-4. Also, Lin et al. *J Exp Med* **188**:199-204 (1998) studied rejection of heart allografts in CD28-deficient mice. H-2(q) hearts were transplanted into allogeneic wild-type or CD28-deficient mice (H-2(b)). Graft rejection was delayed in CD28-deficient compared with wild-type mice. Treatment of wild-type recipients with CTLA-4-immunoglobulin (Ig), or with anti-B7-1 plus anti-B7-2 mAbs significantly prolonged allograft survival. In contrast, treatment of CD28-deficient mice with CTLA-4-Ig, anti-B7-1 plus anti-B7-2 mAbs, or a blocking anti-CTLA-4 mAb induced acceleration of allograft rejection. This increased rate of graft rejection

was associated with more severe mononuclear cell infiltration and enhanced levels of IFN-gamma and IL-6 transcripts in donor hearts of untreated wild-type and CTLA-4-Ig- or anti-CTLA-4 mAb-treated CD28-deficient mice. Thus, the negative regulatory role of CTLA-4 extends beyond its potential ability to prevent CD28 activation through ligand competition. Even in the absence of CD28, CTLA-4 plays an inhibitory role in the regulation of allograft rejection.

Also, further characterization of the expression of CTLA-4 has been investigated. For example, Alegre et al. *J Immunol* **157**:4762-70 (1996) proposed that surface CTLA-4 is rapidly internalized, which may explain the low levels of expression generally detected on the cell surface. They concluded that both CD28 and IL-2 play important roles in the up-regulation of CTLA-4 expression. In addition, the cell surface accumulation of CTLA-4 appeared to be primarily regulated by its rapid endocytosis. Also, Castan et al. *Immunology* **90**:265-71 (1997) based on *in situ* immunohistological analyses of the expression of CTLA-4, suggested that germinal center T cells, which were CTLA-4 positive, could be important to immune regulation.

Accordingly, in view of the broad and pivotal role that CTLA-4 appears to possess in immune responsiveness, it would be desirable to generate antibodies to CTLA-4 that can be utilized effectively in immunotherapy. Moreover, it would be desirable to generate antibodies against CTLA-4 that can be utilized in chronic diseases in which repeat administrations of the antibodies are required.

BRIEF DESCRIPTION OF THE DRAWING FIGURES

Figure 1 provides a series of nucleotide and an amino acid sequences of heavy chain and kappa light chain immunoglobulin molecules in accordance with the invention: 4.1.1 (Figure 1A), 4.8.1 (Figure 1B), 4.14.3 (Figure 1C), 6.1.1 (Figure 1D), 3.1.1 (Figure 1E), 4.10.2 (Figure 1F), 2.1.3 (Figure 1G), 4.13.1 (Figure 1H), 11.2.1 (Figure 1I), 11.6.1 (Figure 1J), 11.7.1 (Figure 1K), 12.3.1.1 (Figure 1L), and 12.9.1.1 (Figure 1M).

Figure 2 provides a sequence alignment between the predicted heavy chain amino acid sequences from the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 3.1.1,

4.10.2, 4.13.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1 and the germline DP-50 (3-33) amino acid sequence. Differences between the DP-50 germline sequence and that of the sequence in the clones are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibodies as shaded.

Figure 3 provides a sequence alignment between the predicted heavy chain amino acid sequence of the clone 2.1.3 and the germline DP-65 (4-31) amino acid sequence. Differences between the DP-65 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 4 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clones 4.1.1, 4.8.1, 4.14.3, 6.1.1, 4.10.2, and 4.13.1 and the germline A27 amino acid sequence. Differences between the A27 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined. Apparent deletions in the CDR1s of clones 4.8.1, 4.14.3, and 6.1.1 are indicated with "0s".

Figure 5 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clones 3.1.1, 11.2.1, 11.6.1, and 11.7.1 and the germline 012 amino acid sequence. Differences between the 012 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 6 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 2.1.3 and the germline A10/A26 amino acid sequence. Differences between the A10/A26 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 7 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 12.3.1 and the germline A17

amino acid sequence. Differences between the A17 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 8 provides a sequence alignment between the predicted kappa light chain amino acid sequence of the clone 12.9.1 and the germline A3/A19 amino acid sequence. Differences between the A3/A19 germline sequence and that of the sequence in the clone are indicated in bold. The Figure also shows the positions of the CDR1, CDR2, and CDR3 sequences of the antibody as underlined.

Figure 9 provides a summary of N-terminal amino acid sequences generated through direct protein sequencing of the heavy and light chains of the antibodies.

Figure 10 provides certain additional characterizing information about certain of the antibodies in accordance with the invention. In Figure 10A, data related to clones 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.14.3, and 6.1.1 is summarized. Data related to concentration, isoelectric focusing (IEF), SDS-PAGE, size exclusion chromatography, liquid chromatography/mass spectroscopy (LCMS), mass spectroscopy (MALDI), light chain N-terminal sequences is provided. Additional detailed information related to IEF is provided in Figure 10B; related to SDS-PAGE is provided in 10C; and SEC of the 4.1.1 antibody in 10D.

Figure 11 shows the expression of B7-1 and B7-2 on Raji cells using anti-CD80-PE and anti-CD86-PE mAbs.

Figure 12 shows the concentration dependent enhancement of IL-2 production in the T cell blast/Raji assay induced by anti-CTLA-4 blocking antibodies (BNI3, 4.1.1, 4.8.1, and 6.1.1).

Figure 13 shows the concentration dependent enhancement of IFN-γ production in the T cell blast/Raji assay induced by anti-CTLA-4 blocking antibodies (BNI3, 4.1.1, 4.8.1, and 6.1.1)(same donor T cells).

Figure 14 shows the mean enhancement of IL-2 production in T cells from 6 donors induced by anti-CTLA-4 blocking antibodies in the T cell blast/Raji assay.

Figure 15 shows the mean enhancement of IFN-γ production in T cells from 6 donors induced by anti-CTLA-4 blocking antibodies in the T cell blast/Raji assay.

Figure 16 shows the enhancement of IL-2 production in hPBMC from 5 donors induced by anti-CTLA-4 blocking mAbs as measured at 72 hours after stimulation with SEA.

Figure 17 shows the enhancement of IL-2 production in whole blood from 3 donors induced by anti-CTLA-4 blocking mAbs as measured at 72 and 96 hours after stimulation with SEA.

Figure 18 shows the inhibition of tumor growth with an anti-murine CTLA-4 antibody in a murine fibrosarcoma tumor model.

Figure 19 shows enhancement of IL-2 production induced by anti-CTLA4 antibodies (4.1.1 and 11.2.1) of the invention in a 72 hour T blast/Raji and Superantigen (whole blood and peripheral blood mononuclear cells from 6 donors) assays.

Figure 20 shows dose dependent enhancement of IL-2 production induced by anti-CTLA4 antibodies (4.1.1 and 11.2.1) of the invention in a 72 hour T blast/Raji assay.

Figure 21 shows dose dependent enhancement of IL-2 production induced by anti-CTLA4 antibodies (4.1.1 and 11.2.1) of the invention in a 72 hour Superantigen whole blood assay stimulated with 100 ng/ml superantigen.

Figure 22 provides a series of additional nucleotide and amino acid sequences of the following anti-CTLA-4 antibody chains: full length 4.1.1 heavy chain (cDNA 22(a), genomic 22(b), and amino acid 22(c)), full length aglycosylated 4.1.1 heavy chain (cDNA 22(d) and amino acid 22(e)), 4.1.1 light chain (cDNA 22(f) and amino acid 22(g)), full length 4.8.1 heavy chain (cDNA 22(h) and amino acid 22(i)), 4.8.1 light chain (cDNA 22(j) and amino acid 22(k)), full length 6.1.1 heavy chain (cDNA 22(l) and amino acid 22(m)), 6.1.1 light chain (cDNA 22(n) and amino acid 22(o)), full length 11.2.1 heavy chain (cDNA 22(p) and amino acid 22(q)), and 11.2.1 light chain (cDNA 22 (r) and amino acid 22(s)).

SUMMARY OF THE INVENTION

In accordance with a first aspect of the present invention, there is provided an antibody that is capable of binding CTLA-4, comprising a heavy chain variable region amino acid sequence that comprises a contiguous amino acid sequence from within an FR1 sequence through an FR3 sequence that is encoded by a human V_H3-33 family gene and that comprises at least one of the amino acid substitutions in the CDR1 sequences, CDR2 sequences, or framework sequences shown in Figure 2. In a preferred embodiment, the amino acid sequence comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, and SEQ ID NO:70. In another preferred embodiment, the antibody further comprises a light chain variable region amino acid sequence comprising a sequence selected from the group consisting of a sequence comprising SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, and SEQ ID NO:71.

In accordance with a second aspect of the present invention, there is provided an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO:1 and a light chain variable amino acid sequence comprising SEQ ID NO:14.

In accordance with a third aspect of the present invention, there is provided an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO:2 and a light chain variable amino acid sequence comprising SEQ ID NO:15.

In accordance with a fourth aspect of the present invention, there is provided an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO:4 and a light chain variable amino acid sequence comprising SEQ ID NO:17.

In accordance with a fifth aspect of the present invention, there is provided an isolated human monoclonal antibody that is capable of binding to CTLA-4. In a preferred embodiment, antibody is capable of competing for binding with CTLA-4 with an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. In another preferred embodiment, the antibody possesses a substantially similar binding specificity to CTLA-4 as an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. In another preferred embodiment, the antibody is selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. In another preferred embodiment, the antibody is not cross reactive with CTLA-4 from lower mammalian species, preferably the lower mammalian species comprises mouse, rat, and rabbit and more preferably mouse and rat. In another preferred embodiment, the antibody is cross reactive with CTLA-4 from primates, preferably the primates comprise cynomolgous and rhesus monkeys. In another preferred embodiment, the antibody possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than about 100:1 and preferably about 500:1 or greater. In another preferred embodiment, the binding affinity of the antibody is about 10^{-9} M or greater and preferably about 10^{-10} M or greater. In another preferred embodiment, the antibody inhibits binding between CTLA-4 and B7-2 with an IC_{50} of lower than about 100 nM and preferably lower than about 0.38 nM. In another preferred embodiment, the antibody inhibits binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 100 nM or greater and preferably lower than about 0.50 nM. In another preferred embodiment, the antibody enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 3846 pg/ml or greater. In another preferred embodiment, the antibody enhances IFN-γ production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 1233 pg/ml or greater. In another preferred embodiment, the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or greater. In another preferred embodiment, the antibody enhances IL-2

production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or preferably 1500 pg/ml or greater or by greater than about 30% or preferably 50% relative to control.

In accordance with a sixth aspect of the present invention, there is provided a humanized antibody that possesses a substantially similar binding specificity to CTLA-4 as an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. In a preferred embodiment, the antibody is not cross reactive with CTLA-4 from lower mammalian species, preferably the lower mammalian species comprises mouse, rat, and rabbit and preferably mouse and rat. In another preferred embodiment, the antibody is cross reactive with CTLA-4 from primates, preferably the primates comprise cynomolgous and rhesus monkeys. In another preferred embodiment, the antibody possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than about 100:1 and preferably about 500:1 or greater. In another preferred embodiment, the binding affinity of the antibody is about 10^{-9} M or greater and preferably about 10^{-10} M or greater. In another preferred embodiment, the antibody inhibits binding between CTLA-4 and B7-2 with an IC_{50} of lower than about 100 nM and preferably lower than about 0.38 nM. In another preferred embodiment, the antibody inhibits binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 100 nM or greater and preferably lower than about 0.50 nM. In another preferred embodiment, the antibody enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 3846 pg/ml or greater. In another preferred embodiment, the antibody enhances IFN-γ production in a T cell blast/Raji assay by about 500 pg/ml or greater and preferably by about 1233 pg/ml or greater. In another preferred embodiment, the antibody induces IL-2 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or greater. In another preferred embodiment, the antibody enhances IL-2 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or preferably 1500 pg/ml or greater or by greater than about 30% or preferably 50% relative to control.

In accordance with a seventh aspect of the present invention, there is provided an antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded by a human V_H 3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, the CDR1, CDR2, and CDR3 sequences being independently selected from the CDR1, CDR2, and CDR3 sequences illustrated in Figure 2. In a preferred embodiment, the antibody of Claim 32, further comprising any of the somatic mutations to the FR1, FR2, and FR3 sequences as illustrated in Figure 2.

In accordance with an eighth aspect of the present invention, there is provided an antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences encoded by a human V_H 3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, which antibody has the following properties: a binding affinity for CTLA-4 of about 10^{-9} or greater; inhibits binding between CTLA-4 and B7-1 with an IC_{50} of about 100 nM or lower; inhibits binding between CTLA-4 and B7-2 with an IC_{50} of about 100 nM or lower; and enhances cytokine production in an assay of human T cells by 500 pg/ml or greater.

In accordance with a ninth aspect of the present invention, there is provided an antibody that binds to CTLA-4, comprising a heavy chain amino acid sequence comprising FR1, FR2, and FR3 sequences operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence independently selected from the CDR1, CDR2, and CDR3 sequences illustrated in Figures 2 and 3, which antibody has the following properties: a binding affinity for CTLA-4 of about 10^{-9} or greater; inhibits binding between CTLA-4 and B7-1 with an IC_{50} of about 100 nM or lower; inhibits binding between CTLA-4 and B7-2 with an IC_{50} of about 100 nM or lower; and enhances cytokine production in an assay of human T cells by 500 pg/ml or greater.

In accordance with a tenth aspect of the present invention, there is provided a cell culture system for assaying T cell stimulation, comprising a culture of human T cell blasts co-cultured with a Raji cell line. In a preferred embodiment, the T cell blasts are washed prior to culture with the Raji cell line.

In accordance with an eleventh aspect of the present invention, there is provided an assay for measuring T cell stimulation, comprising: providing a culture of human T cell blasts and a Raji cell line; contacting the culture with an agent; and measuring cytokine production by the culture.

In accordance with an twelfth aspect of the present invention, there is provided a functional assay for screening a moiety for T cell stimulatory function, comprising: providing a culture of human T cell blasts and a Raji cell line; contacting the culture with the moiety; and assessing cytokine production by the culture.

In accordance with a thirteenth aspect of the present invention, there is provided a T cell stimulatory assay for CTLA-4 inhibitory function, comprising contacting a culture comprising human T cell blasts and a Raji cell line with an agent and assessing cytokine production by the culture.

In accordance with a fourteenth aspect of the present invention, there is provided a method for screening an agent for T cell stimulatory activity, comprising: contacting the agent with a cell culture comprising human T cell blasts and a Raji cell line; and assessing cytokine production by the culture.

In each of the tenth through the fourteenth aspects of the present invention, in a preferred embodiment, the T cell blasts are washed prior to culture with the Raji cell line. In another preferred embodiment, the cytokine is IL-2 or IFN-γ. In a preferred embodiment, cytokine production is measured in supernatant isolated from the culture. In a preferred embodiment, the agent is an antibody and preferably binds to CTLA-4.

In accordance with a fifteenth aspect of the present invention, there is provided an assay for measuring T cell stimulation, comprising: providing a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A; contacting the culture with an agent; and measuring cytokine production by the cell population.

In accordance with a sixteenth aspect of the present invention, there is provided a functional assay for screening a moiety for T cell stimulatory function, comprising: providing a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus

enterotoxin A; contacting the culture with the moiety; and assessing cytokine production by the cell population.

In accordance with a seventeenth aspect of the present invention, there is provided a T cell stimulatory assay for CTLA-4 inhibitory function, comprising contacting a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A with an agent and assessing cytokine production by the cell population.

In accordance with an eighteenth aspect of the present invention, there is provided a method for screening an agent for T cell stimulatory activity, comprising: contacting the agent with a population of human peripheral blood mononuclear cells or human whole blood stimulated with staphylococcus enterotoxin A; and assessing cytokine production by the cell population.

In each of the fifteenth through the eighteenth aspects of the present invention, in a preferred embodiment, the cytokine is IL-2. In another preferred embodiment, cytokine production is measured in supernatant isolated from the culture. In a preferred embodiment, the agent is an antibody and preferably binds to CTLA-4.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In accordance with the present invention, there are provided fully human monoclonal antibodies against human CTLA-4. Nucleotide sequences encoding and amino acid sequences comprising heavy and light chain immunoglobulin molecules, particularly sequences corresponding to a contiguous heavy and light chain sequences from FR1 and CDR1 through CDR3 and FR4, are provided. Further provided are antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein. Hybridomas expressing such immunoglobulin molecules and monoclonal antibodies are also provided.

Definitions

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. *See e.g.,* Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference. The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

The term "isolated polynucleotide" as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the "isolated polynucleotide" (1) is not associated with all or a portion of a polynucleotide in which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence.

The term "isolated protein" referred to herein means a protein of cDNA, recombinant RNA, or synthetic origin or some combination thereof, which by virtue of its origin, or source of derivation, the "isolated protein" (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, e.g. free of murine proteins, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

The term "polypeptide" as used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein, fragments, and analogs are species of the polypeptide genus. Preferred polypeptides in accordance with the invention comprise the human heavy chain immunoglobulin molecules and the human kappa light chain immunoglobulin molecules represented in Figure 1, as well as antibody molecules formed by combinations comprising the heavy chain immunoglobulin molecules with light chain immunoglobulin molecules, such as the kappa light chain immunoglobulin molecules, and vice versa, as well as fragments and analogs thereof.

The term "naturally-occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory or otherwise is naturally-occurring.

The term "operably linked" as used herein refers to positions of components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

The term "control sequence" as used herein refers to polynucleotide sequences which are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

The term "oligonucleotide" referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. Preferably oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g. for probes; although oligonucleotides may be double stranded, e.g. for use in the construction of a gene mutant.

Oligonucleotides of the invention can be either sense or antisense oligonucleotides.

The term "naturally occurring nucleotides" referred to herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phoshoraniladate, phosphoroamidate, and the like. *See e.g.*, LaPlanche et al. *Nucl. Acids Res.* **14**:9081 (1986); Stec et al. *J. Am. Chem. Soc.* **106**:6077 (1984); Stein et al. *Nucl. Acids Res.* **16**:3209 (1988); Zon et al. *Anti-Cancer Drug Design* **6**:539 (1991); Zon et al. *Oligonucleotides and Analogues: A Practical Approach*, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec et al. U.S. Patent No. 5,151,510; Uhlmann and Peyman *Chemical Reviews* **90**:543 (1990), the disclosures of which are hereby incorporated by reference. An oligonucleotide can include a label for detection, if desired.

The term "selectively hybridize" referred to herein means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments thereof in accordance with the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High stringency conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, and fragments of the invention and a nucleic acid sequence of interest will be at least 80%, and more typically with preferably increasing homologies of at least 85%, 90%, 95%, 99%, and 100%. Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for

maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Alternatively and preferably, two protein sequences (or polypeptide sequences derived from them of at least 30 amino acids in length) are homologous, as this term is used herein, if they have an alignment score of at more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. *See* Dayhoff, M.O., in *Atlas of Protein Sequence and Structure*, pp. 101-110 (Volume 5, National Biomedical Research Foundation (1972)) and Supplement 2 to this volume, pp. 1-10. The two sequences or parts thereof are more preferably homologous if their amino acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program. The term "corresponds to" is used herein to mean that a polynucleotide sequence is homologous (i.e., is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence, or that a polypeptide sequence is identical to a reference polypeptide sequence. In contradistinction, the term "complementary to" is used herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For illustration, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA".

The following terms are used to describe the sequence relationships between two or more polynucleotide or amino acid sequences: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 18 nucleotides or 6 amino acids in length, frequently at least 24 nucleotides or 8 amino acids in length, and often at least 48 nucleotides or 16 amino acids in length. Since two polynucleotides or amino acid sequences may each (1)

comprise a sequence (i.e., a portion of the complete polynucleotide or amino acid sequence) that is similar between the two molecules, and (2) may further comprise a sequence that is divergent between the two polynucleotides or amino acid sequences, sequence comparisons between two (or more) molecules are typically performed by comparing sequences of the two molecules over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window", as used herein, refers to a conceptual segment of at least 18 contiguous nucleotide positions or 6 amino acids wherein a polynucleotide sequence or amino acid sequence may be compared to a reference sequence of at least 18 contiguous nucleotides or 6 amino acid sequences and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions, deletions, substitutions, and the like (i.e., gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* **2**:482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* **48**:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (U.S.A.)* **85**:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, (Genetics Computer Group, 575 Science Dr., Madison, Wis.), Geneworks, or MacVector software packages), or by inspection, and the best alignment (i.e., resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected.

The term "sequence identity" means that two polynucleotide or amino acid sequences are identical (i.e., on a nucleotide-by-nucleotide or residue-by-residue basis) over the comparison window. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) or residue occurs in both

sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the comparison window (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The terms "substantial identity" as used herein denotes a characteristic of a polynucleotide or amino acid sequence, wherein the polynucleotide or amino acid comprises a sequence that has at least 85 percent sequence identity, preferably at least 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 18 nucleotide (6 amino acid) positions, frequently over a window of at least 24-48 nucleotide (8-16 amino acid) positions, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the comparison window. The reference sequence may be a subset of a larger sequence.

As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. *See Immunology - A Synthesis* (2nd Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference. Stereoisomers (e.g., D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α-, α-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids include: 4-hydroxyproline, γ -carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ-N-methylarginine, and other similar amino acids and imino acids (e.g., 4-hydroxyproline). In the polypeptide notation used herein, the lefthand direction is the amino terminal direction and the righthand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

Similarly, unless specified otherwise, the lefthand end of single-stranded polynucleotide sequences is the 5' end; the lefthand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the RNA and which are 5' to the 5' end of the RNA transcript are referred to as "upstream sequences"; sequence regions on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences".

As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity, and most preferably at least 99 percent sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamic-aspartic, and asparagine-glutamine.

As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being encompassed by the present invention, providing that the variations in the

amino acid sequence maintain at least 75%, more preferably at least 80%, 90%, 95%, and most preferably 99%. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into families: (1) acidic=aspartate, glutamate; (2) basic=lysine, arginine, histidine; (3) non-polar=alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar=glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. More preferred families are: serine and threonine are aliphatic-hydroxy family; asparagine and glutamine are an amide-containing family; alanine, valine, leucine and isoleucine are an aliphatic family; and phenylalanine, tryptophan, and tyrosine are an aromatic family. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide derivative. Assays are described in detail herein. Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie et al. *Science* **253**:164 (1991). Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the invention.

Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the naturally-occurring sequence (preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et at. *Nature* **354**:105 (1991), which are each incorporated herein by reference.

The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence deduced, for example, from a full-length cDNA sequence. Fragments typically are at least 5, 6, 8 or 10 amino acids long, preferably at least 14 amino acids long, more preferably at least 20 amino acids long, usually at least 50 amino acids long, and even more preferably at least 70 amino acids long. The term "analog" as used herein refers to polypeptides which are comprised of a segment of at least 25 amino acids that has substantial identity to a portion of a deduced amino acid sequence and which has at least one of the following properties: (1) specific binding to CTLA-4, under suitable

binding conditions, (2) ability to block CTLA-4 binding with its receptors, or (3) ability to inhibit CTLA-4 expressing cell growth *in vitro* or *in vivo*. Typically, polypeptide analogs comprise a conservative amino acid substitution (or addition or deletion) with respect to the naturally-occurring sequence. Analogs typically are at least 20 amino acids long, preferably at least 50 amino acids long or longer, and can often be as long as a full-length naturally-occurring polypeptide.

Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drus with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics". Fauchere, *J. Adv. Drug Res.* **15**:29 (1986); Veber and Freidinger *TINS* p.392 (1985); and Evans et al. *J. Med. Chem.* **30**:1229 (1987), which are incorporated herein by reference. Such compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biochemical property or pharmacological activity), such as human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: --CH_2NH--, --CH_2S--, --CH_2-CH_2--, --CH=CH- (cis and trans), --$COCH_2$--, --$CH(OH)CH_2$--, and --CH_2SO--, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch *Ann. Rev. Biochem.* **61**:387 (1992), incorporated herein by reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

"Antibody" or "antibody peptide(s)" refer to an intact antibody, or a binding fragment thereof that competes with the intact antibody for specific binding. Binding fragments are produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact antibodies. Binding fragments include Fab, Fab', $F(ab')_2$, Fv, and single-chain antibodies. An antibody other than a "bispecific" or "bifunctional" antibody is understood to have each of its binding sites identical. An antibody substantially inhibits adhesion of a receptor to a counterreceptor when an excess of antibody reduces the quantity of receptor bound to counterreceptor by at least about 20%, 40%, 60% or 80%, and more usually greater than about 85% (as measured in an *in vitro* competitive binding assay).

The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. An antibody is said to specifically bind an antigen when the dissociation constant is ≤ 1 μM, preferably ≤ 100 nM and most preferably ≤ 10 nM.

The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

As used herein, the terms "label" or "labeled" refers to incorporation of a detectable marker, e.g., by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In certain situations, the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the

following: radioisotopes or radionuclides (*e.g.*, ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I), fluorescent labels (*e.g.*, FITC, rhodamine, lanthanide phosphors), enzymatic labels (*e.g.*, horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

The term "pharmaceutical agent or drug" as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient. Other chemistry terms herein are used according to conventional usage in the art, as exemplified by *The McGraw-Hill Dictionary of Chemical Terms* (Parker, S., Ed., McGraw-Hill, San Francisco (1985)), incorporated herein by reference).

The term "antineoplastic agent" is used herein to refer to agents that have the functional property of inhibiting a development or progression of a neoplasm in a human, particularly a malignant (cancerous) lesion, such as a carcinoma, sarcoma, lymphoma, or leukemia. Inhibition of metastasis is frequently a property of antineoplastic agents.

As used herein, "substantially pure" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more preferably more than about 85%, 90%, 95%, and 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot

be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

The term patient includes human and veterinary subjects.

Antibody Structure

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. *See generally, Fundamental Immunology* Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site.

Thus, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions

of Kabat *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk *J. Mol. Biol.* **196**:901-917 (1987); Chothia et al. *Nature* **342**:878-883 (1989).

A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. *See, e.g.*, Songsivilai & Lachmann *Clin. Exp. Immunol.* **79**: 315-321 (1990), Kostelny et al. *J. Immunol.* **148**:1547-1553 (1992). In addition, bispecific antibodies may be formed as "diabodies" (Holliger et al. "'Diabodies': small bivalent and bispecific antibody fragments" *PNAS USA* **90**:6444-6448 (1993)) or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" *EMBO J* **10**:3655-3659 (1991) and Traunecker et al. "Janusin: new molecular design for bispecific reagents" *Int J Cancer Suppl* **7**:51-52 (1992)). Production of bispecific antibodies can be a relatively labor intensive process compared with production of conventional antibodies and yields and degree of purity are generally lower for bispecific antibodies. Bispecific antibodies do not exist in the form of fragments having a single binding site (e.g., Fab, Fab', and Fv).

Human Antibodies and Humanization of Antibodies

Human antibodies avoid certain of the problems associated with antibodies that possess murine or rat variable and/or constant regions. The presence of such murine or rat derived proteins can lead to the rapid clearance of the antibodies or can lead to the generation of an immune response against the antibody by a patient. In order to avoid the utilization of murine or rat derived antibodies, it has been postulated that one can develop humanized antibodies or generate fully human antibodies through the introduction of human antibody function into a rodent so that the rodent would produce antibodies having fully human sequences.

Human Antibodies

The ability to clone and reconstruct megabase-sized human loci in YACs and to introduce them into the mouse germline provides a powerful approach to elucidating the functional components of very large or crudely mapped loci as well as generating useful models of human disease. Furthermore, the utilization of such technology for substitution of mouse loci with their human equivalents could provide unique insights into the expression and regulation of human gene products during development, their communication with other systems, and their involvement in disease induction and progression.

An important practical application of such a strategy is the "humanization" of the mouse humoral immune system. Introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig genes have been inactivated offers the opportunity to study the mechanisms underlying programmed expression and assembly of antibodies as well as their role in B-cell development. Furthermore, such a strategy could provide an ideal source for production of fully human monoclonal antibodies (Mabs) an important milestone towards fulfilling the promise of antibody therapy in human disease. Fully human antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized Mabs and thus to increase the efficacy and safety of the administered antibodies. The use of fully human antibodies can be expected to provide a substantial advantage in the treatment of chronic and recurring human diseases, such as inflammation, autoimmunity, and cancer, which require repeated antibody administrations.

One approach towards this goal was to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci in anticipation that such mice would produce a large repertoire of human antibodies in the absence of mouse antibodies. Large human Ig fragments would preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse machinery for antibody diversification and selection and the lack of immunological tolerance

to human proteins, the reproduced human antibody repertoire in these mouse strains should yield high affinity antibodies against any antigen of interest, including human antigens. Using the hybridoma technology, antigen-specific human Mabs with the desired specificity could be readily produced and selected.

This general strategy was demonstrated in connection with our generation of the first XenoMouse™ strains as published in 1994. *See* Green et al. *Nature Genetics* 7:13-21 (1994). The XenoMouse™ strains were engineered with yeast artificial chromosomes (YACs) containing 245 kb and 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus, respectively, which contained core variable and constant region sequences. *Id.* The human Ig containing YACs proved to be compatible with the mouse system for both rearrangement and expression of antibodies and were capable of substituting for the inactivated mouse Ig genes. This was demonstrated by their ability to induce B-cell development, to produce an adult-like human repertoire of fully human antibodies, and to generate antigen-specific human Mabs. These results also suggested that introduction of larger portions of the human Ig loci containing greater numbers of V genes, additional regulatory elements, and human Ig constant regions might recapitulate substantially the full repertoire that is characteristic of the human humoral response to infection and immunization. The work of Green et al. was recently extended to the introduction of greater than approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and kappa light chain loci, respectively, to produce XenoMouse™ mice. *See* Mendez et al. *Nature Genetics* **15**:146-156 (1997), Green and Jakobovits *J. Exp. Med.* **188**:483-495 (1998), and U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996, the disclosures of which are hereby incorporated by reference.

Such approach is further discussed and delineated in U.S. Patent Application Serial Nos. 07/466,008, filed January 12, 1990, 07/610,515, filed November 8, 1990, 07/919,297, filed July 24, 1992, 07/922,649, filed July 30,

1992, filed 08/031,801, filed March 15,1993, 08/112,848, filed August 27, 1993, 08/234,145, filed April 28, 1994, 08/376,279, filed January 20, 1995, 08/430, 938, April 27, 1995, 08/464,584, filed June 5, 1995, 08/464,582, filed June 5, 1995, 08/463,191, filed June 5, 1995, 08/462,837, filed June 5, 1995, 08/486,853, filed June 5, 1995, 08/486,857, filed June 5, 1995, 08/486,859, filed June 5, 1995, 08/462,513, filed June 5, 1995, 08/724,752, filed October 2, 1996, and 08/759,620, filed December 3, 1996. *See also* Mendez et al. *Nature Genetics* **15**:146-156 (1997) and Green and Jakobovits *J. Exp. Med.* **188**:483-495 (1998). *See also* European Patent No., EP 0 463 151 B1, grant published June 12, 1996, International Patent Application No., WO 94/02602, published February 3, 1994, International Patent Application No., WO 96/34096, published October 31, 1996, and WO 98/24893, published June 11, 1998. The disclosures of each of the above-cited patents, applications, and references are hereby incorporated by reference in their entirety.

In an alternative approach, others, including GenPharm International, Inc., have utilized a "minilocus" approach. In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more V_H genes, one or more D_H genes, one or more J_H genes, a mu constant region, and a second constant region (preferably a gamma constant region) are formed into a construct for insertion into an animal. This approach is described in U.S. Patent No. 5,545,807 to Surani et al. and U.S. Patent Nos. 5,545,806, 5,625,825, 5,625,126, 5,633,425, 5,661,016, 5,770,429, 5,789,650, and 5,814,318 each to Lonberg and Kay, U.S. Patent No. 5,591,669 to Krimpenfort and Berns, U.S. Patent Nos. 5,612,205, 5,721,367, 5,789,215 to Berns et al., and U.S. Patent No. 5,643,763 to Choi and Dunn, and GenPharm International U.S. Patent Application Serial Nos. 07/574,748, filed August 29, 1990, 07/575,962, filed August 31, 1990, 07/810,279, filed December 17, 1991, 07/853,408, filed March 18, 1992, 07/904,068, filed June 23, 1992, 07/990,860, filed December 16, 1992, 08/053,131, filed April 26, 1993, 08/096,762, filed July 22, 1993, 08/155,301, filed November 18, 1993, 08/161,739, filed December 3, 1993, 08/165,699, filed December 10, 1993, 08/209,741, filed March 9, 1994, the disclosures of

which are hereby incorporated by reference. *See also* European Patent No. 0 546 073 B1, International Patent Application Nos. WO 92/03918, WO 92/22645, WO 92/22647, WO 92/22670, WO 93/12227, WO 94/00569, WO 94/25585, WO 96/14436, WO 97/13852, and WO 98/24884, the disclosures of which are hereby incorporated by reference in their entirety. *See further* Taylor et al., 1992, Chen et al., 1993, Tuaillon et al., 1993, Choi et al., 1993, Lonberg et al., (1994), Taylor et al., (1994), and Tuaillon et al., (1995), Fishwild et al., (1996), the disclosures of which are hereby incorporated by reference in their entirety.

The inventors of Surani et al., cited above and assigned to the Medical Research Counsel (the "MRC"), produced a transgenic mouse possessing an Ig locus through use of the minilocus approach. The inventors on the GenPharm International work, cited above, Lonberg and Kay, following the lead of the present inventors, proposed inactivation of the endogenous mouse Ig locus coupled with substantial duplication of the Surani et al. work.

An advantage of the minilocus approach is the rapidity with which constructs including portions of the Ig locus can be generated and introduced into animals. Commensurately, however, a significant disadvantage of the minilocus approach is that, in theory, insufficient diversity is introduced through the inclusion of small numbers of V, D, and J genes. Indeed, the published work appears to support this concern. B-cell development and antibody production of animals produced through use of the minilocus approach appear stunted. Therefore, research surrounding the present invention has consistently been directed towards the introduction of large portions of the Ig locus in order to achieve greater diversity and in an effort to reconstitute the immune repertoire of the animals.

Human anti-mouse antibody (HAMA) responses have led the industry to prepare chimeric or otherwise humanized antibodies. While chimeric antibodies have a human constant region and a murine variable region, it is expected that certain human anti-chimeric antibody (HACA) responses will be observed, particularly in chronic or multi-dose utilizations of the antibody.

Thus, it would be desirable to provide fully human antibodies against CTLA-4 in order to vitiate concerns and/or effects of HAMA or HACA response.

Humanization and Display Technologies

As was discussed above in connection with human antibody generation, there are advantages to producing antibodies with reduced immunogenicity. To a degree, this can be accomplished in connection with techniques of humanization and display techniques using appropriate libraries. It will be appreciated that murine antibodies or antibodies from other species can be humanized or primatized using techniques well known in the art. *See e.g.*, Winter and Harris *Immunol Today* 14:43-46 (1993) and Wright et al. *Crit. Reviews in Immunol.* 12125-168 (1992). The antibody of interest may be engineered by recombinant DNA techniques to substitute the CH1, CH2, CH3, hinge domains, and/or the framework domain with the corresponding human sequence (*see* WO 92/02190 and U.S. Patent Nos. 5,530,101, 5,585,089, 5,693,761, 5,693,792, 5,714,350, and 5,777,085). Also, the use of Ig cDNA for construction of chimeric immunoglobulin genes is known in the art (Liu et al. *P.N.A.S.* **84**:3439 (1987) and *J.Immunol.***139**:3521 (1987)). mRNA is isolated from a hybridoma or other cell producing the antibody and used to produce cDNA. The cDNA of interest may be amplified by the polymerase chain reaction using specific primers (U.S. Pat. Nos. 4,683,195 and 4,683,202). Alternatively, a library is made and screened to isolate the sequence of interest. The DNA sequence encoding the variable region of the antibody is then fused to human constant region sequences. The sequences of human constant regions genes may be found in Kabat et al. (1991) Sequences of Proteins of Immunological Interest, N.I.H. publication no. 91-3242. Human C region genes are readily available from known clones. The choice of isotype will be guided by the desired effector functions, such as complement fixation, or activity in antibody-dependent cellular cytotoxicity. Preferred isotypes are IgG1, IgG2, IgG3 and IgG4. Particularly preferred isotypes for antibodies of the invention are IgG2 and IgG4. Either of the human light chain constant regions, kappa or

lambda, may be used. The chimeric, humanized antibody is then expressed by conventional methods.

Antibody fragments, such as Fv, $F(ab')_2$ and Fab may be prepared by cleavage of the intact protein, e.g. by protease or chemical cleavage. Alternatively, a truncated gene is designed. For example, a chimeric gene encoding a portion of the $F(ab')_2$ fragment would include DNA sequences encoding the CH1 domain and hinge region of the H chain, followed by a translational stop codon to yield the truncated molecule.

In one approach, consensus sequences encoding the heavy and light chain J regions may be used to design oligonucleotides for use as primers to introduce useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments. C region cDNA can be modified by site directed mutagenesis to place a restriction site at the analogous position in the human sequence.

Expression vectors include plasmids, retroviruses, cosmids, YACs, EBV derived episomes, and the like. A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C region, and also at the splice regions that occur within the human CH exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The resulting chimeric antibody may be joined to any strong promoter, including retroviral LTRs, e.g. SV-40 early promoter, (Okayama et al. *Mol. Cell. Bio.* 3:280 (1983)), Rous sarcoma virus LTR (Gorman et al. *P.N.A.S.* 79:6777 (1982)), and moloney murine leukemia virus LTR (Grosschedl et al. *Cell* 41:885 (1985)); native lg promoters, etc.

Further, human antibodies or antibodies from other species can be generated through display-type technologies, including, without limitation, phage display, retroviral display, ribosomal display, and other techniques, using techniques well known in the art and the resulting molecules can be subjected to

additional maturation, such as affinity maturation, as such techniques are well known in the art. Wright and Harris, *supra.*, Hanes and Plucthau *PNAS USA* **94**:4937-4942 (1997) (ribosomal display), Parmley and Smith *Gene* **73**:305-318 (1988) (phage display), Scott *TIBS* **17**:241-245 (1992), Cwirla et al. *PNAS USA* **87**:6378-6382 (1990), Russel et al. *Nucl. Acids Research* **21**:1081-1085 (1993), Hoganboom et al. *Immunol. Reviews* **130**:43-68 (1992), Chiswell and McCafferty *TIBTECH* **10**:80-84 (1992), and U.S. Patent No. 5,733,743. If display technologies are utilized to produce antibodies that are not human, such antibodies can be humanized as described above.

Using these techniques, antibodies can be generated to CTLA-4 expressing cells, CTLA-4 itself, forms of CTLA-4, epitopes or peptides thereof, and expression libraries thereto (*see e.g.* U.S. Patent No. 5,703,057) which can thereafter be screened as described above for the activities described above.

Additional Criteria for Antibody Therapeutics

As will be appreciated, it is generally not desirable to kill CTLA-4 expressing cells. Rather, one generally desires to simply inhibit CTLA-4 binding with its ligands to mitigate T cell down regulation. One of the major mechanisms through which antibodies kill cells is through fixation of complement and participation in CDC. The constant region of an antibody plays an important role in connection with an antibody's ability to fix complement and participate in CDC. Thus, generally one selects the isotype of an antibody to either provide the ability of complement fixation, or not. In the case of the present invention, generally, as mentioned above, it is generally not preferred to utilize an antibody that kills the cells. There are a number of isotypes of antibodies that are capable of complement fixation and CDC, including, without limitation, the following: murine IgM, murine IgG2a, murine IgG2b, murine IgG3, human IgM, human IgG1, and human IgG3. Those isotypes that do not include, without limitation, human IgG2 and human IgG4.

It will be appreciated that antibodies that are generated need not initially possess a particular desired isotype but, rather, the antibody as generated can

possess any isotype and the antibody can be isotype switched thereafter using conventional techniques that are well known in the art. Such techniques include the use of direct recombinant techniques (*see e.g.*, U.S. Patent No. 4,816,397), cell-cell fusion techniques (*see e.g.*, U.S. Patent Application No. 08/730,639, filed October 11, 1996), among others.

In the cell-cell fusion technique, a myeloma or other cell line is prepared that possesses a heavy chain with any desired isotype and another myeloma or other cell line is prepared that possesses the light chain. Such cells can, thereafter, be fused and a cell line expressing an intact antibody can be isolated.

By way of example, the majority of the CTLA-4 antibodies discussed herein are human anti-CTLA-4 IgG2 antibody. Since such antibodies possess desired binding to the CTLA-4 molecule, any one of such antibodies can be readily isotype switched to generate a human IgG4 isotype, for example, while still possessing the same variable region (which defines the antibody's specificity and some of its affinity).

Accordingly, as antibody candidates are generated that meet desired "structural" attributes as discussed above, they can generally be provided with at least certain additional "functional" attributes that are desired through isotype switching.

Design and Generation of Other Therapeutics

In accordance with the present invention and based on the activity of the antibodies that are produced and characterized herein with respect to CTLA-4, the design of other therapeutic modalities including other antibodies, other antagonists, or chemical moieties other than antibodies is facilitated. Such modalities include, without limitation, antibodies having similar binding activity or functionality, advanced antibody therapeutics, such as bispecific antibodies, immunotoxins, and radiolabeled therapeutics, generation of peptide therapeutics, gene therapies, particularly intrabodies, antisense therapeutics, and small molecules. Furthermore, as discussed above, the effector function of the

antibodies of the invention may be changed by isotype switching to an IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM for various therapeutic uses.

In connection with the generation of advanced antibody therapeutics, where complement fixation is a desirable attribute, it may be possible to sidestep the dependence on complement for cell killing through the use of bispecifics, immunotoxins, or radiolabels, for example.

In connection with bispecific antibodies, bispecific antibodies can be generated that comprise (i) two antibodies one with a specificity to CTLA-4 and another to a second molecule that are conjugated together, (ii) a single antibody that has one chain specific to CTLA-4 and a second chain specific to a second molecule, or (iii) a single chain antibody that has specificity to CTLA-4 and the other molecule. Such bispecific antibodies can be generated using techniques that are well known for example, in connection with (i) and (ii) *see e.g.,* Fanger et al. *Immunol Methods* 4:72-81 (1994) and Wright and Harris, *supra.* and in connection with (iii) *see e.g.,* Traunecker et al. *Int. J. Cancer (Suppl.)* 7:51-52 (1992).

In addition, "Kappabodies" (Ill et al. "Design and construction of a hybrid immunoglobulin domain with properties of both heavy and light chain variable regions" *Protein Eng* 10:949-57 (1997)), "Minibodies" (Martin et al. "The affinity-selection of a minibody polypeptide inhibitor of human interleukin-6" *EMBO J* 13:5303-9 (1994)), "Diabodies" (Holliger et al. "'Diabodies': small bivalent and bispecific antibody fragments" *PNAS USA* 90:6444-6448 (1993)), or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" *EMBO J* 10:3655-3659 (1991) and Traunecker et al. "Janusin: new molecular design for bispecific reagents" *Int J Cancer Suppl* 7:51-52 (1992)) may also be prepared.

In connection with immunotoxins, antibodies can be modified to act as immunotoxins utilizing techniques that are well known in the art. *See e.g.,* Vitetta *Immunol Today* 14:252 (1993). *See also* U.S. Patent No. 5,194,594. In connection with the preparation of radiolabeled antibodies, such modified antibodies can also be readily prepared utilizing techniques that are well known

in the art. *See e.g.*, Junghans et al. in *Cancer Chemotherapy and Biotherapy* 655-686 (2d edition, Chafner and Longo, eds., Lippincott Raven (1996)). *See also* U.S. Patent Nos. 4,681,581, 4,735,210, 5,101,827, 5,102,990 (RE 35,500), 5,648,471, and 5,697,902. Each of immunotoxins and radiolabeled molecules would be likely to kill cells expressing CTLA-4, and particularly those cells in which the antibodies of the invention are effective.

In connection with the generation of therapeutic peptides, through the utilization of structural information related to CTLA-4 and antibodies thereto, such as the antibodies of the invention (as discussed below in connection with small molecules) or screening of peptide libraries, therapeutic peptides can be generated that are directed against CTLA-4. Design and screening of peptide therapeutics is discussed in connection with Houghten et al. *Biotechniques* 13:412-421 (1992), Houghten *PNAS USA* 82:5131-5135 (1985), Pinalla et al. *Biotechniques* 13:901-905 (1992), Blake and Litzi-Davis *BioConjugate Chem.* 3:510-513 (1992). Immunotoxins and radiolabeled molecules can also be prepared, and in a similar manner, in connection with peptidic moieties as discussed above in connection with antibodies.

Important information related to the binding of an antibody to an antigen can be gleaned through phage display experimentation. Such experiments are generally accomplished through panning a phage library expressing random peptides for binding with the antibodies of the invention to determine if peptides can be isolated that bind. If successful, certain epitope information can be gleaned from the peptides that bind.

In general, phage libraries expressing random peptides can be purchased from New England Biolabs (7-mer and 12-mer libraries, Ph.D.-7 Peptide 7-mer Library Kit and Ph.D.-12 Peptide 12-mer Library Kit, respectively) based on a bacteriophage M13 system. The 7-mer library represents a diversity of approximately 2.0×10^9 independent clones, which represents most, if not all, of the $20^7 = 1.28 \times 10^9$ possible 7-mer sequences. The 12-mer library contains approximately 1.9×10^9 independent clones and represents only a very small sampling of the potential sequence space of $20^{12} = 4.1 \times 10^{15}$ 12-mer sequences. Each of 7-mer and 12-mer libraries are panned or screened in accordance with

the manufacturer's recommendations in which plates were coated with an antibody to capture the appropriate antibody (a goat anti-human IgG Fc for an IgG antibody for example) followed by washing. Bound phage are eluted with 0.2 M glycine-HCl, pH 2.2. After 3 rounds of selection/amplification at constant stringency (0.5% Tween), through use of DNA sequencing, one can characterize clones from the libraries that are reactive with one or more of the antibodies. Reactivity of the peptides can be determined by ELISA. For an additional discussion of epitope analysis of peptides *see also* Scott, J.K. and Smith, G.P. *Science* 249:386-390 (1990); Cwirla et al. *PNAS USA* 87:6378-6382 (1990); Felici et al. *J. Mol. Biol.* 222:301-310 (1991), and Kuwabara et al. *Nature Biotechnology* 15:74-78 (1997).

The design of gene and/or antisense therapeutics through conventional techniques is also facilitated through the present invention. Such modalities can be utilized for modulating the function of CTLA-4. In connection therewith the antibodies of the present invention facilitate design and use of functional assays related thereto. A design and strategy for antisense therapeutics is discussed in detail in International Patent Application No. WO 94/29444. Design and strategies for gene therapy are well known. However, in particular, the use of gene therapeutic techniques involving intrabodies could prove to be particularly advantageous. *See e.g.,* Chen et al. *Human Gene Therapy* 5:595-601 (1994) and Marasco *Gene Therapy* 4:11-15 (1997). General design of and considerations related to gene therapeutics is also discussed in International Patent Application No. WO 97/38137. Genetic materials encoding an antibody of the invention (such as the 4.1.1, 4.8.1, or 6.1.1, or others) may be included in a suitable expression system (whether viral, attenuated viral, non-viral, naked, or otherwise) and administered to a host for *in vivo* generation of the antibody in the host.

Small molecule therapeutics can also be envisioned in accordance with the present invention. Drugs can be designed to modulate the activity of CTLA-4 based upon the present invention. Knowledge gleaned from the structure of the CTLA-4 molecule and its interactions with other molecules in accordance with the present invention, such as the antibodies of the invention, CD28, B7,

B7-1, B7-2, and others can be utilized to rationally design additional therapeutic modalities. In this regard, rational drug design techniques such as X-ray crystallography, computer-aided (or assisted) molecular modeling (CAMM), quantitative or qualitative structure-activity relationship (QSAR), and similar technologies can be utilized to focus drug discovery efforts. Rational design allows prediction of protein or synthetic structures which can interact with the molecule or specific forms thereof which can be used to modify or modulate the activity of CTLA-4. Such structures can be synthesized chemically or expressed in biological systems. This approach has been reviewed in Capsey et al. *Genetically Engineered Human Therapeutic Drugs* (Stockton Press, NY (1988)). Indeed, the rational design of molecules (either peptides, peptidomimetics, small molecules, or the like) based upon known, or delineated, structure-activity relationships with other molecules (such as antibodies in accordance with the invention) has become generally routine. *See, e.g.,* Fry et al. "Specific, irreversible inactivation of the epidermal growth factor receptor and erbB2, by a new class of tyrosine kinase inhibitor" *Proc Natl Acad Sci U S A* **95**:12022-7 (1998); Hoffman et al. "A model of Cdc25 phosphatase catalytic domain and Cdk-interaction surface based on the presence of a rhodanese homology domain" *J Mol Biol* **282**:195-208 (1998); Ginalski et al. "Modelling of active forms of protein kinases: p38--a case study" *Acta Biochim Pol* **44**:557-64 (1997); Jouko et al. "Identification of csk tyrosine phosphorylation sites and a tyrosine residue important for kinase domain structure" *Biochem J* **322**:927-35 (1997); Singh et al. "Structure-based design of a potent, selective, and irreversible inhibitor of the catalytic domain of the erbB receptor subfamily of protein tyrosine kinases" *J Med Chem* **40**:1130-5 (1997); Mandel et al. "ABGEN: a knowledge-based automated approach for antibody structure modeling" *Nat Biotechnol* **14**:323-8 (1996); Monfardini et al. "Rational design, analysis, and potential utility of GM-CSF antagonists" *Proc Assoc Am Physicians* **108**:420-31 (1996); Furet et al. "Modelling study of protein kinase inhibitors: binding mode of staurosporine and origin of the selectivity of CGP 52411" *J Comput Aided Mol Des* **9**:465-72 (1995).

Further, combinatorial libraries can be designed and sythesized and used in screening programs, such as high throughput screening efforts.

Therapeutic Administration and Formulations

It will be appreciated that administration of therapeutic entities in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, PA (1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present invention, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. *See also* Powell et al. "Compendium of excipients for parenteral formulations" *PDA J Pharm Sci Technol.* 52:238-311 (1998) and the citations therein for additional information related to excipients and carriers well known to pharmaceutical chemists.

Preparation of Antibodies

Antibodies in accordance with the invention are preferably prepared through the utilization of a transgenic mouse that has a substantial portion of the human antibody producing genome inserted but that is rendered deficient in the production of endogenous, murine, antibodies. Such mice, then, are capable of

producing human immunoglobulin molecules and antibodies and are deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving the same are disclosed in the patents, applications, and references disclosed in the Background, herein. In particular, however, a preferred embodiment of transgenic production of mice and antibodies therefrom is disclosed in U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996, the disclosure of which is hereby incorporated by reference. *See also* Mendez et al. *Nature Genetics* 15:146-156 (1997), the disclosure of which is hereby incorporated by reference.

Through use of such technology, we have produced fully human monoclonal antibodies to a variety of antigens. Essentially, we immunize XenoMouse™ lines of mice with an antigen of interest, recover lymphatic cells (such as B-cells) from the mice that express antibodies, fuse such recovered cells with a myeloid-type cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. We utilized these techniques in accordance with the present invention for the preparation of antibodies specific to CTLA-4. Herein, we describe the production of multiple hybridoma cell lines that produce antibodies specific to CTLA-4. Further, we provide a characterization of the antibodies produced by such cell lines, including nucleotide and amino acid sequence analyses of the heavy and light chains of such antibodies.

The antibodies derived from hybridoma cell lines discussed herein are designated 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1. Each of the antibodies produced by the aforementioned cell lines are either fully human IgG2 or IgG4 heavy chains with human kappa light chains. In general, antibodies in accordance with the invention possess very high affinities, typically possessing Kd's of from about 10^{-9} through about 10^{-11} M, when measured by either solid phase or solution phase.

As will be appreciated, antibodies in accordance with the present invention can be expressed in cell lines other than hybridoma cell lines.

Sequences encoding the cDNAs or genomic clones for the particular antibodies can be used for transformation of a suitable mammalian or nonmammalian host cells. Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (which patents are hereby incorporated herein by reference). The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, particle bombardment, encapsulation of the polynucleotide(s) in liposomes, peptide conjugates, dendrimers, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, NSO_0, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines. Non-mammalian cells including but not limited to bacterial, yeast, insect, and plants can also be used to express recombinant antibodies. Site directed mutagenesis of the antibody CH2 domain to eliminate glycosylation may be preferred in order to prevent changes in either the immunogenicity, pharmacokinetic, and/or effector functions resulting from non-human glycosylation. The expression methods are selected by determining which system generates the highest expression levels and produce antibodies with constitutive CTLA-4 binding properties.

Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, the glutamine sythetase and DHFR gene expression systems are common approaches for enhancing expression under certain

conditions. High expressing cell clones can be identified using conventional techniques, such as limited dilution cloning and Microdrop technology. The GS system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 89303964.4.

Antibodies of the invention can also be produced transgenically through the generation of a mammal or plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom. In connection with the transgenic production in mammals, antibodies can be produced in, and recovered from, the milk of goats, cows, or other mammals. *See, e.g.*, U.S. Patent Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957.

Antibodies in accordance with the present invention have been analyzed structurally and functionally. In connection with the structures of the antibodies, amino acid sequences of the heavy and kappa light chains have been predicted based on cDNA sequences obtained through RT-PCR of the hybridomas. *See* Examples 3 and 4 and Figures 1-8. N-terminal sequencing of the antibodies was also conducted in confirmation of the results discussed in Examples 3 and 4. *See* Example 5 and Figure 9. Kinetic analyses of the antibodies were conducted to determine affinities. *See* Example 2. Antibodies in accordance with the invention (and particularly the 4.1.1, 4.8.1, and 6.1.1 antibodies of the invention) have high affinities (4.1.1:1.63 X 10^{10} 1/M; 4.8.1:3.54 X 10^{10} 1/M; and 6.1.1:7.2 X 10^{9} 1/M). Further, antibodies were analyzed by isoelectric focusing (IEF), reducing gel electrophoresis (SDS-PAGE), size exclusion chromatography, liquid chromatography/mass spectroscopy, and mass spectroscopy and antibody production by the hybridomas was assessed. *See* Example 6 and Figure 10.

In connection with functional analysis of antibodies in accordance with the present invention, such antibodies proved to be potent inhibitors of CTLA-4 and its binding to its ligands of the B7 family of molecules. For example, antibodies in accordance with the present invention were demonstrated to block CTLA-4 binding to either B7-1 or B7-2. *See* Example 7. Indeed, many of the

antibodies in accordance with the invention possess nanomolar and subnanomolar IC_{50}s with respect to inhibiting CTLA-4 binding to B7-1 and B7-2. Further, antibodies of the invention possess excellent selectivity for CTLA-4 as compared to CD28, CD44, B7-2, or hIgG1. *See* Example 8. Selectivity is a ratio that reflects the degree of preferential binding of a molecule with a first agent as compared to the molecules binding with a second, and optionally other molecules. Herein, selectivity refers to the degree of preferential binding of an antibody of the invention to CTLA-4 as compared to the antibody's binding to other molecules such as CD28, CD44, B7-2, or hIgG1. Selectivity values of antibodies of the invention greater than 500:1 are common. Antibodies of the invention have also been demonstrated to induce or enhance expression of certain cytokines (such as IL-2 and IFN-γ) by cultured T cells in a T cell blast model. *See* Examples 9 and 10 and Figures 12-17. Further, it is expected that antibodies of the invention will inhibit the growth of tumors in appropriate *in vivo* tumor models. The design of which models are discussed in Example 11 and 12.

The results demonstrated in accordance with the present invention indicate that antibodies of the present invention possess certain qualities that may make the present antibodies more efficacious than current therapeutic antibodies against CTLA-4.

In particular, the 4.1.1, 4.8.1, and 6.1.1 antibodies of the invention possess highly desirable properties. Their structural characteristics, functions, or activities provide criteria that facilitate the design or selection of additional antibodies or other molecules as discussed above. Such criteria include one or more of the following:

Ability to compete for binding to CTLA-4 with one or more of the antibodies of the invention;

Similar binding specificity to CTLA-4 as one or more of the antibodies of the invention;

A binding affinity for CTLA-4 of about 10^{-9} M or greater and preferably of about 10^{-10}M or greater;

Does not cross react with lower mammalian CTLA-4, including, preferably, mouse, rat, or rabbit and preferably mouse or rat CTLA-4;

Cross reacts with primate CTLA-4, including, preferably, cynomolgous and rhesus CTLA-4;

A selectivity for CTLA-4 over CD28, B7-2, CD44, or hIgG1 of at least about 100:1 or greater and preferably of about 300, 400, or 500:1 or greater;

An IC_{50} in blocking CTLA-4 binding to B7-2 of about 100 nM or lower and preferably 5, 4, 3, 2, 1, 0.5, or 0.38 nM or lower;

An IC_{50} in blocking CTLA-4 binding to B7-1 of about of about 100 nM or lower and preferably 5, 4, 3, 2, 1, 0.5, or 0.50 nM or lower;

An enhancement of cytokine production in one or more *in vitro* assays, for example:

An enhancement of IL-2 production in a T cell blast/Raji assay of about 500 pg/ml or greater and preferably 750, 1000, 1500, 2000, 3000, or 3846 pg/ml or greater;

An enhancement of IFN-γ production in a T cell blast/Raji assay of about 500 pg/ml or greater and preferably 750, 1000, or 1233 pg/ml or greater; or

An enhancement of IL-2 production in a hPBMC or whole blood superantigen assay of about 500 pg/ml or greater and preferably 750, 1000, 1200, or 1511 pg/ml or greater. Expressed another way, it is desirable that IL-2 production is enhanced by about 30, 35, 40, 45, 50 percent or more relative to control in the assay.

It is expected that antibodies (or molecules designed or synthesized therefrom) having one or more of these properties will possess similar efficacy to the antibodies described in the present invention.

The desirable functional properties discussed above can often result from binding to and inhibition of CTLA4 by a molecule (i.e., antibody, antibody fragment, peptide, or small molecule) in a similar manner as an antibody of the invention (i.e., binding to the same or similar epitope of the CTLA4 molecule).

The molecule may either be administered directly (i.e., direct administration to a patient of such molecules). Or, alternatively, the molecule may be "administered" indirectly (i.e., a peptide or the like that produces an immune response in a patient (similar to a vaccine) wherein the immune response includes the generation of antibodies that bind to the same or similar epitope or an antibody or fragment that is produced *in situ* after administration of genetic materials that encode such antibodies or fragments thereof which bind to the same or similar epitope). Thus, it will be appreciated that the epitope on CTLA4 to which antibodies of the invention bind to can be useful in connection with the preparation and/or design of therapeutics in accordance with the invention. In drug design, negative information is often useful as well (i.e., the fact that an antibody which binds to CTLA4 does not appear to bind to an epitope that acts as an inhibitor of CTLA4 is useful). Thus, the epitope to which antibodies of the invention bind that do not lead to the desired functionality can also be very useful. Accordingly, also contemplated in accordance with the present invention are molecules (and particularly antibodies) that bind to the same or similar epitopes as antibodies of the invention.

In addition to the fact that antibodies of the invention and the epitopes to which they bind are contemplated in accordance with the invention, we have conducted some preliminary epitope mapping studies of certain antibodies in accordance with the invention and particularly the 4.1.1 and the 11.2.1 antibodies of the invention.

As a first step, we conducted BIAcore competition studies to generate a rough map of binding as between certain antibodies of the invention in connection with their ability to compete for binding to CTLA4. To this end, CTLA4 was bound to a BIAcore chip and a first antibody, under saturating conditions, was bound thereto and competition of subsequent secondary antibodies binding to CTLA4 was measured. This technique enabled generation of a rough map in to which families of antibodies can be classified.

Through this process, we determined that the certain antibodies in accordance with the invention could be categorized as falling into the following epitopic categories:

Category	Antibodies	Competition for CTLA4 Binding
A	BO1M*	Freely cross-compete with one another; cross-compete with category B; some cross-competition with category D
	BO2M**	
B	4.1.1	Freely cross-compete with one another; cross-compete with category A, C and D.
	4.13.1	
C	6.1.1	Freely cross-compete with one another; cross-compete with category B and category D
	3.1.1	
	4.8.1	
	11.2.1	
	11.6.1	
	11.7.1	
D	4.14.3	Cross-compete with category C and B; some cross-competition with category A
E	4.9.1	BNI3 blocks 4.9.1 binding to CTLA4 but not the reverse
	BNI3***	

(*) (**) Available from Biostride.

(***) Available from Pharmingen.

As a next step, we endeavored to determine if the antibodies of the invention recognized a linear epitope on CTLA4 under reducing and non-reducing conditions on Western blots. We observed that none of the 4.1.1, 3.1.1, 11.7.1, 11.6.1, or 11.2.1 antibodies of the invention appeared to recognize a reduced form of CTLA4 on Western blot. Accordingly, it appeared likely that the epitope to which each of these antibodies bound was not a linear epitope but more likely was a conformational epitope the structure of which may have been abrogated under reducing conditions.

Therefore, we sought to determine whether we could learn about residues within the CTLA4 molecule that are important for binding of antibodies of the invention. One manner that we utilized was to conduct kinetic assessments of off-rates as between human CTLA4 and two highly conserved primate CTLA4 molecules (cynomologous and marmoset CTLA4). BIAcore

studies demonstrated that the 4.1.1 antibody of the invention bound to human, cynomologous, and marmoset CTLA4 at the same rate. However, with respect to off-rates (affinity), the 4.1.1 antibody had the highest affinity (slowest off-rate) for human, a faster off-rate with cynomologous, and a much faster off-rate for marmoset. The 11.2.1 antibody of the invention, on the other hand, binds to human, cynomologous, and marmoset CTLA4 at the about the same rate and has about the same relative off-rate for each of the three. This information further indicates that the 4.1.1 and 11.2.1 antibodies of the invention bind to different epitopes on CTLA4.

To further study the epitope to which the category B and C antibodies of the invention bind, we conducted certain site directed mutagenesis studies. Marmoset CTLA4 possesses two important changes at residues 105 and 106 relative to human CTLA4. Such differences are a leucine to methionine change at residue 105 and a glycine to serine change at residue 106. Accordingly, we mutated cDNA encoding human CTLA4 to encode a mutated CTLA4 having the L105M and G106S changes. The homologue replacement mutant CTLA4 did not effect binding of a B7.2-IgG1 fusion protein. Further, binding with the 11.2.1 antibody of the invention was not effected. However, such molecule was significantly inhibited in its ability to bind with the 4.1.1 antibody of the invention (similar to marmoset). Next, we mutated a cDNA encoding marmoset CTLA4 to create a mutant marmoset CTLA4 having a S106G change. Such change resulted in restoration of stable binding between the 4.1.1 antibody and the marmoset CTLA4 mutant. In addition, we mutated a cDNA encoding marmoset CTLA4 to create a mutant marmoset CTLA4 having a M105L change. Such change partially restored binding between the 4.1.1 antibody and the mutant CTLA4.

Each of the category B through D antibodies of the invention appear to possess similar functional properties and appear to have the potential to act as strong anti-CTLA4 therapeutic agents. Further, each of the molecules certain cross-competition in their binding for CTLA4. However, as will be observed from the above discussion, each of the molecules in the different categories appear to bind to separate conformational epitopes on CTLA4.

From the foregoing, it will be appreciated that the epitope information discussed above indicates that antibodies (or other molecules, as discussed above) that cross-compete with antibodies of the invention will likely have certain therapeutic potential in accordance with the present invention. Further, it is expected that antibodies (or other molecules, as discussed above) that cross-compete with antibodies of the invention (i.e., cross-compete with category B, C and/or D antibodies) will likely have certain additional therapeutic potential in accordance with the present invention. Additionally, it is expected that antibodies (or other molecules, as discussed above) that cross-compete with antibodies of the invention (i.e., cross-compete with category B, C and/or D antibodies) and that (i) are not reduced in their binding to marmoset CTLA4 (similar to the 11.2.1 antibody) or (ii) are reduced in their binding to marmoset CTLA4 (similar to the 4.1.1 antibody) will likely have certain additional therapeutic potential in accordance with the present invention. Antibodies (or other molecules, as discussed above) that compete with categories A and E may also have certain therapeutic potential.

EXAMPLES

The following examples, including the experiments conducted and results achieved are provided for illustrative purposes only and are not to be construed as limiting upon the present invention.

EXAMPLE 1

Generation of Anti-CTLA-4-Antibody Producing Hybridomas

Antibodies of the invention were prepared, selected, and assayed in accordance with the present Example.

Antigen Preparation: Three distinct immunogens were prepared for immunization of the XenoMouse™ mice: (i) a CTLA-4-IgG fusion protein, (ii) a CTLA-4 peptide, and (iii) 300.19 murine lymphoma cells transfected with

a mutant of CTLA-4 (Y201V) that is constitutively expressed on the cell surface.

(i) ***CTLA-4-IgG1 Fusion Protein:***

Expression Vector Construction:

The cDNA encoding the mature extracellular domain of CTLA-4 was PCR amplified from human thymus cDNA library (Clontech) using primers designed to published sequence (*Eur. J Immunol* **18**:1901-1905 (1988)). The fragment was directionally subcloned into pSR5, a Sindbis virus expression plasmid (InVitrogen), between the human oncostatin M signal peptide and human IgG gamma 1 (IgG1) CH1/CH2/CH3 domains. The fusion protein does not contain a hinge domain but contains cysteine 120 in the extracellular domain of CTLA-4 to form a covalent dimer. The resulting vector was called CTLA-4-IgG1/pSR5. The complete CTLA-4-IgG1 cDNA in the vector was sequence confirmed in both strands. The amino acid sequence the CTLA4-Ig protein is shown below. The mature extracellular domain for CD44 was PCR amplified from human lymphocyte library (Clontech) and subcloned into pSinRep5 to generate a control protein with the identical IgG1 tail.

OM-CTLA4-IgG1 Fusion Protein:

MGVLLTQRTLLSLVLALLFPSMASMAMHVAQPAVVLASSRGIASFVC
EYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICT
GTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIY
VIDPEPCPDSDLEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPTPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Underlined: signal peptide

Bold: CTLA4 extracellular domain

The cDNAs for mature extracellular domain of CD28 were PCR amplified from human lymphocyte library (Clontech) and then subcloned into pCDM8 (*J. Immunol.* **151**: 5261-71 (1993)) to produce a human IgG1 fusion protein containing both thrombin cleavage and hinge regions. Marmoset, Cynomologous, and Rhesus CTLA4 were cloned from mRNA isolated from PHA stimulated PBMCs using standard techniques of degenerate PCR. Sequencing demonstrated that rhesus and cynomologous amino acid sequence were identical with three differences from mature human CTLA4 extracellular domain (S13N, I17T and L105M). Marmoset demonstrated ten amino acid differences from the mature human CTLA4 extracellular domain (V21A, V33I, A41T, A51G, 54I, S71F, Q75K, T88M, L105M and G106S). Site directed mutagenesis was used to make single point mutations of all amino acids different in marmoset CTLA4 to map amino acids important for interation of the antibodies with human CTLA4-IgG. Mutations of human and marmoset CTLA-IgG for epitope mapping were generated by matchmaker site-directed mutagenesis (Promega). The IgG fusion proteins were produced by transient transfection of Cos7 cells and purified using standard Protein A techniques. Mutant CTLA4-IgG proteins were evaluated for binding to antibodies by immunoblotting and using BIAcore analyses.

Recombinant Protein Expression/Purification:

Recombinant sindbis virus was generated by electroporating (Gibco) Baby Hamster Kidney cells with SP6 *in vitro* transcribed CTLA-4-IgG1/pSR5 mRNA and DH-26S helper mRNA as described by InVitrogen. Forty eight hours later recombinant virus was harvested and titered for optimal protein expression in Chinese hamster ovary cells (CHO-K1). CHO-K1 cells were cultured in suspension in DMEM/F12 (Gibco) containing 10% heat-inactivated

fetal bovine serum (Gibco), non-essential amino acids (Gibco), 4mM glutamine (Gibco), penicillin/streptomycin (Gibco), 10mM Hepes pH 7.5 (Gibco). To produce CTLA-4-IgG, the CHO-K1 cells were resuspended at $1x10^7$ cells/ml in DMEM/F12 and incubated with sindbis virus for one hour at room temperature. Cells were then diluted to $1x10^6$/ml in DMEM/F12 containing 1% fetal bovine serum depleted of bovine IgG using protein A sepharose (Pharmacia), non-essential amino acids, 4mM glutamine, 12.5mM Hepes pH 7.5, and penicillin/streptomycin. Forty eight hours post-infection cells were pelleted and conditioned media was harvested and supplemented with complete protease inhibitor tablets (Boehringer Mannheim), pH adjusted to 7.5, and filtered 0.2μ (Nalgene). FPLC (Pharmacia) was used to affinity purify the fusion protein using a 5ml protein A HiTrap column (Pharmacia) at a 10ml/min flow rate. The column was washed with 30 bed volumes of PBS and eluted with 0.1M glycine/HCl pH 2.8 at 1ml/min. Fractions (1ml) were immediately neutralized to pH 7.5 with Tris pH 9. The fractions containing CTLA-4-IgG1 were identified by SDS-PAGE and then concentrated using centriplus 50 (Amicon) before applying to sepharose 200 column (Pharmacia) at 1ml/min using PBS as the solvent. Fractions containing CTLA-4-IgG1 were pooled, sterile filtered 0.2μ (Millipore), aliquoted and frozen at -80°C. CD44-IgG1 was expressed and purified using the same methods. CD28-IgG was purified from conditioned media from transiently transfected Cos7 cells.

Characterization CTLA-4-IgG1:

The purified CTLA-4-IgG1 migrated as a single band on SDS-PAGE using colloidal coomassie staining (Novex). Under non-reducing conditions CTLA-4-IgG1 was a dimer (100kDa), that reduced to a 50kDa monomer when treated with 50mM DTT. Amino acid sequencing of the purified CTLA-4-IgG1 in solution confirmed the N-terminus of CTLA-4 (MHVAQPAVVLAS), and that the oncostatin-M signal peptide was cleaved from the mature fusion protein . The CTLA-4-IgG1 bound to immobilized B7.1-IgG in a concentration dependent manner and the binding was blocked by a hamster-anti-human anti-

CTLA-4 antibody (BNI3: PharMingen). The sterile CTLA-4-IgG was endotoxin free and quantitated by OD280 using 1.4 as the extinction coefficient. The yield of purified CTLA-4-IgG ranged between 0.5-3mgs/liter of CHO-K1 cells.

(ii) ***CTLA-4 Peptide:***

The following CTLA-4 peptide was prepared as described below:

NH_2:MHVAQPAVVLASSRGIASFVCEYASPGKATEVRVTVLRQADSQVT EVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICK VELMYPPPYYLGIGNGTQIYVIDPEPC-$CONH_2$

Abbreviations/Materials:

NMP, N-Methylpyrrolidinone; TFE, 2,2,2-Trifluoroethanol; DCM, Dichloromethane; FMOC, Fluorenyl Methoxycarbonyl. All reagents were supplied by Perkin Elmer, with the following exceptions: TFE, Aldrich Chemical, FMOC-PAL-PEG resin, Perseptive Biosystems. Fmoc-Arg(PMC)-OH, FMOC-Asn(Trt)-OH, FMOC-Asp(tBu)-OH, FMOC-Cys(Trt)-OH, FMOC-Glu(tBu)-OH, FMOC-Gln(Trt)-OH, FMOC-His(Boc)-OH, FMOC-Lys(BOC)-OH, FMOC-Ser(tBu)-OH, FMOC-Thr(tBu)-OH and FMOC-Tyr(tBu)-OH were used for those amino acids requiring side chain protecting groups

Peptide Synthesis:

Peptide synthesis was performed on a Perkin-Elmer 431A, retrofitted with feedback monitoring via UV absorbance at 301nm (Perkin-Elmer Model 759A detector). The peptide sequence was assembled on a FMOC-PAL-PEG resin using conditional double coupling cycles. Forced double couplings were performed at cycles 10,11,18,19,20 and 28 through 33. The resin was washed with a 50% mixture of DCM and TFE at the completion of each acylation cycle,

followed by capping of unreacted amino groups with acetic anhydride in NMP. Resin was removed from the reactor after completing cycle 49 and the remainder continued to completion. Peptide cleavage from the resin was performed using Reagent K (King et al. *International Journal of Protein and Peptide Research* **36**:255-266 (1990)) for 6 hours on 415mg of resin affording 186mg crude CTLA-4 peptide.

Peptide Characterization:

25mg aliquots of the crude CTLA-4 peptide were dissolved in 5ml 6M Guanidine HCl/100mM K_2PO_3 at pH6.4 and eluted over a Pharmacia Hi Load Superdex 75 16/60 column (16mm x 600mm, 120ml bed volume) with 2M Guanidine.HCl / 100mM K_2PO_3 at pH6.4 at 2 ml / min for 180 minutes collecting 5 ml fractions. The fractions were analyzed by loading 1.7µl of fractions onto a NuPAGE Laemeli gel running with MES running buffer and visualizing via Daichii silver stain protocol. Those fractions exhibiting a molecular weight of 12 KDa, as judged versus molecular weight standards, were pooled together and stored at 4°C. The combined fractions were analyzed by UV and gel electrophoresis. Amino acid sequencing was performed by absorbing a 100 microliter sample in a ProSorb cartridge (absorbed onto a PVDF membrane) and washing to remove the buffer salts. Sequencing was performed on an Applied Biosystems 420. The expected N-terminal sequence (M H V A Q P A V V L A) was observed. Immunoblotting demonstrated that the peptide was recognized by the BNI3 anti-human CTLA-4 (PharMingen). To desalt, an aliquot containing 648µg of material was placed in 3500 Da MWCO dialysis tubing and dialyzed against 0.1% TFA / H2O at 4°C for 9 days with stirring. The entire contents of the dialysis bag was lyophilyzed to a powder.

(iii) *300.19 cells transfected with CTLA-4 (Y201V)*

The full length CTLA-4 cDNA was PCR amplified from human thymus cDNA library (Stratagene) and subcloned into pIRESneo (Clontech). A mutation of CTLA-4 that results in constitutive cell surface expression was introduced using MatchMaker Mutagenesis System (Promega). Mutation of tyrosine, Y201 to valine inhibits binding of the adaptin protein AP50 that is responsible for the rapid internalization of CTLA-4 (Chuang et al. *J. Immunol.* **159**:144-151 (1997)). Mycoplasma-free 300.19 murine lymphoma cells were cultured in RPMI-1640 containing 10% fetal calf serum, non-essential amino acids, penicillin/streptomycin, 2mM glutamine, 12.5mM Hepes pH 7.5, and 25uM beta-mercaptoethanol. Cells were electroporated ($3x10^6$/0.4ml serum free RPMI) in a 1ml chamber with 20ug CTLA-4-Y201V/pIRESneo using 200V/1180uF (Gibco CellPorator). Cells were rested for 10 minutes and then 8mls of prewarmed complete RPMI media. At 48 hours cells were diluted to 0.5 $x10^6$/ml in complete RPMI media containing 1mg/ml G418 (Gibco). Resistant cells were expanded and shown to express CTLA-4 on the cell surface using the BNI3 antibody conjugated with phycoerythrin (PharMingen). High level expressing cells were isolated by sterile sorting.

Immunization and hybridoma generation: XenoMouse mice (8 to 10 weeks old) were immunized (i) subcutaneously at the base of tails with $1x10^7$ 300.19 cells that were transfected to express CTLA-4 as described above, resuspended in phosphate buffered saline (PBS) with complete Freund's adjuvant, or (ii) subcutaneously at the base of tail with (a) 10 μg the CTLA-4 fusion protein or (b) 10 μg CTLA-4 peptide, emulsified with complete Freund's adjuvant. In each case, the dose was repeated three or four times in incomplete Freund's adjuvant. Four days before fusion, the mice received a final injection of the immunogen or cells in PBS. Spleen and/or lymph node lymphocytes from immunized mice were fused with the [murine non-secretory myeloma P3 cell line] and were subjected to HAT selection as previously described (Galfre, G. and Milstein, C., "Preparation of monoclonal antibodies: strategies and

procedures." *Methods Enzymol.* 73:3-46 (1981)). A large panel of hybridomas all secreting CTLA-4 specific human $IgG_2\kappa$ or $IgG_4\kappa$ (as detected below) antibodies were recovered.

ELISA assay: ELISA for determination of antigen-specific antibodies in mouse serum and in hybridoma supernatants was carried out as described (Coligan et al., Unit 2.1, "Enzyme-linked immunosorbent assays," in *Current protocols in immunology* (1994)) using CTLA-4-Ig fusion protein to capture the antibodies. For animals that are immunized with the CTLA-4-Ig fusion protein, we additionally screen for non-specific reactivity against the human Ig portion of the fusion protein. This is accomplished using ELISA plates coated with human IgG1 as a negative control for specificity.

In a preferred ELISA assay, the following techniques are used:

ELISA plates are coated with 100 µl/well of the antigen in plate coating buffer (0.1 M Carbonate Buffer, pH 9.6 and $NaHCO_3$ (MW 84) 8.4g/L). Plates are then incubated at 4°C overnight. After incubation, coating buffer is removed and the plate is blocked with 200 µl/well blocking buffer (0.5% BSA, 0.1% Tween 20, 0.01% Thimerosal in 1x PBS) and incubated at room temperature for 1 hour. Alternatively, the plates are stored in refrigerator with blocking buffer and plate sealers. Blocking buffer is removed and 50 µl/well of hybridoma supernatant, serum or other hybridoma supernatant (positive control) and HAT media or blocking buffer (negative control) is added. The plates are incubated at room temperature for 2 hours. After incubation, the plate is washed with washing buffer (1x PBS). The detecting antibody (i.e., mouse anti-human IgG2-HRP (SB, #9070-05) for IgG2 antibodies or mouse anti-human IgG4-HRP (SB #9200-05) for IgG4 antibodies) is added at 100µl/well (mouse anti-human IgG2-HRP @ 1:2000 or mouse anti-human IgG4-HRP @ 1:1000 (each diluted in blocking buffer)). The plates are incubated at room temperature for 1 hour and then washed with washing buffer. Thereafter, 100 µl/well of freshly prepared developing solution (10 ml Substrate buffer, 5 mg OPD (o-phenylenediamine, Sigma Cat No. P-7288), and 10 µl 30% H_2O_2 (Sigma)) is added to the wells. The plates are allowed to develop 10-20 minutes, until

negative control wells barely start to show color. Thereafter, 100 µl/well of stop solution (2 M H_2SO_4) is added and the plates are read on an ELISA plate reader at wavelength 490 nm.

Determination of affinity constants of fully human Mabs by BIAcore: Affinity measurement of purified human monoclonal antibodies, Fab fragments, or hybridoma supernatants by plasmon resonance was carried out using the BIAcore 2000 instrument, using general procedures outlined by the manufacturers.

Kinetic analysis of the antibodies was carried out using antigens immobilized onto the sensor surface at a low density. Three surfaces of the BIAcore sensorchip were immobililized with the CTLA-4-Ig fusion protein at a density ranging from approximately 390-900 using CTLA-4-Ig fusion protein at 20 or 50 µg/ml in 10 mM sodium acetate at pH 5.0 using the amine coupling kit supplied by the manufacturer (BIAcore, Inc.). The fourth surface of the BIAcore sensorchip was immobilized with IgG1 (900 RU) and was used as a negative control surface for non-specific binding. Kinetic analysis was performed at a flow rate of 25 or 50 microliters per minute and dissociation (kd or k_{off}) and association (ka or k_{on}) rates were determined using the software provided by the manufacturer (BIA evaluation 3.0) that allows for global fitting calculations.

EXAMPLE 2

Affinity Measurement of Anti-CTLA-4-Antibodies

In the following Table, affinity measurements for certain of the antibodies selected in this manner are provided:

TABLE I

	Solid Phase (by BIAcore)				
Hybridoma	On-rates K_a ($M^{-1}S^{-1}x10^6$)	Off-rates K_d ($S^{-1}x10^{-4}$)	Association Constant KA (1/M)= $k_a/k_d x10^{10}$	Dissociation Constant KD(M)= $K_d/k_a x10^{-10}$	Surface Density [RU]
Moab01	0.68	1.01	0.67	1.48	878.7
	0.70	4.66	0.15	6.68	504.5
	0.77	6.49	0.19	8.41	457.2
	0.60	3.08	0.20	5.11	397.8
4.1.1	1.85	0.72	2.58	0.39	878.7
	1.88	1.21	1.55	0.64	504.5
	1.73	1.54	1.13	0.88	457.2
	1.86	1.47	1.26	0.79	397.8
4.8.1	0.32	0.07	4.46	0.22	878.7
	0.31	0.23	1.33	0.75	504.5
	0.28	0.06	4.82	0.21	397.8
4.14.3	2.81	3.04	0.92	1.08	878.7
	2.88	3.97	0.73	1.38	504.5
	2.84	6.66	0.43	2.35	457.2
	3.17	5.03	0.63	1.58	397.8
6.1.1	0.43	0.35	1.21	0.83	878.7
	0.46	0.90	0.51	1.98	504.5
	0.31	0.51	0.61	1.63	457.2
	0.45	0.79	0.57	1.76	397.8
3.1.1	1.04	0.96	1.07	0.93	878.7
	0.95	1.72	0.55	1.82	504.5
	0.73	1.65	0.44	2.27	457.2
	0.91	2.07	0.44	2.28	397.8
4.9.1	1.55	13.80	0.11	8.94	878.7
	1.43	19.00	0.08	13.20	504.5
	1.35	20.50	0.07	15.20	397.8
4.10.2	1.00	2.53	0.39	2.54	878.7
	0.94	4.30	0.22	4.55	504.5
	0.70	5.05	0.14	7.21	457.2
	1.00	5.24	0.19	5.25	397.8
2.1.3	1.24	9.59	0.13	7.72	878.7
	1.17	13.10	0.09	11.20	504.5
	1.11	13.00	0.09	11.70	397.8
4.13.1	1.22	5.83	0.21	4.78	878.7
	1.29	6.65	0.19	5.17	504.5
	1.23	7.25	0.17	5.88	397.8

As will be observed, antibodies prepared in accordance with the invention possess high affinities and binding constants.

EXAMPLE 3

Structures of Anti-CTLA-4-Antibodies Prepared in Accordance with the Invention

In the following discussion, structural information related to antibodies prepared in accordance with the invention is provided.

In order to analyze structures of antibodies produced in accordance with the invention, we cloned genes encoding the heavy and light chain fragments out of the particular hybridoma. Gene cloning and sequencing was accomplished as follows:

Poly(A)$^+$ mRNA was isolated from approximately 2 X 10^5 hybridoma cells derived from immunized XenoMouse mice using a Fast-Track kit (Invitrogen). The generation of random primed cDNA was followed by PCR. Human V_H or human V_κ family specific variable region primers (Marks et al., "Oligonucleotide primers for polymerase chain reaction amplification of human immunoglobulin variable genes and design of family-specific oligonucleotide probes." *Eur. J. Immunol.* 21:985-991 (1991)) or a universal human V_H primer, MG-30 (CAGGTGCAGCTGGAGCAGTCIGG) was used in conjunction with primers specific for the human Cγ2 constant region (MG-40d; 5'-GCTGAGGGAGTAGAGTCCTGAGGA-3') or Cκ constant region (hκP2; as previously described in Green et al., 1994). Sequences of human Mabs-derived heavy and kappa chain transcripts from hybridomas were obtained by direct sequencing of PCR products generated from poly(A$^+$) RNA using the primers described above. PCR products were also cloned into pCRII using a TA cloning kit (Invitrogen) and both strands were sequenced using

Draft

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Inventor: Todd Butler

ARYL SUBSTITUTED IMIDAZO[4,5-C] PYRIDINE ANTAGONISTS OF THE COMPLEMENT ACTIVATION PATHWAY, FOR USE IN PHARMACEUTICAL COMPOSITIONS AND TREATMENT METHODS

BACKGROUND

The present invention provides novel pharmaceutically active compounds that antagonize the mammalian C3a receptor, and methods of using these compounds to treat chronic inflammatory diseases, including, but not limited to inflammations in the central nervous system (CNS), peripheral nervous system (PNS), lungs, and bone joints.

The human complement system is a ~~complex interaction of components comprising~~ a group of approximately 30 ~~components~~ proteins that work in a coordinated fashion toward host defense and clearance of damaged tissue after trauma. ~~comprising enzymes, plasma and membrane components, factors, regulators, and receptors working together to target invading microorganisms and antigens.~~ (See Grant et al., *Bioorganic & Medicinal ChemistryLetters* 11 (2001) 2817–2820) Complement activation occurs by one of three primary modes known as the "classical" pathway, the "alternative" pathway and the "lectin" pathway. (see, also, e.g., Ember et al. (1997) Immunopharmacology 38:3-15).These pathways are distinguished by the processes that initiate complement activation. The classical pathway is initiated by antibody-antigen complexes or aggregated forms of immunoglobulins.~~;~~ Tthe alternative pathway is initiated ~~in several ways,~~ by a number of stimuli that includeing ~~the spontaneous~~ cleavage of ~~a~~ thioesters, by ~~certain structures~~ moieties present on ~~microbial and~~ cell surfaces, ~~such as~~ that include amino groups or, hydroxyl groups~~., and by water; and t~~ The lectin pathway~~, which is an antibody-independent pathway that~~ is initiated ~~by~~ through the ~~binding of~~ mannan-binding lectin (MBL, also designated mannan-binding protein) ~~to carbohydrates~~ (see, e.g., Thiel et al. (1997) Nature 386:506-510).

In U.S. Patent No. 6,297,024, Hugli et al. disclose that the classical pathway is initiated by the binding of the first complement component (C1) to immune complexes through C1q, a subcomponent involved in binding to antibody. The C1 complex is composed of C1q and two homologous serine proteases, C1r and C1s. After binding to the immune complexes, C1q undergoes a conformational change resulting in the conversions of C1r and C1s to their activated forms. Activated C1s cleaves C4 and C2 to generate a complex of their fragments C4b2a, which in turn cleaves C3 into C3a and C3b. C3b binds to immune complexes.

Gerard et al. disclose that during complement activation, the 74-77 amino acid anaphylatoxins C3a, C4a and C5a are released. These anaphylatoxins mediate a number of inflammatory processes, ~~are potent inflammatory mediators, inducing~~ that include cellular degranulation, smooth muscle contraction, arachidonic acid metabolism, cytokine release, and cellular chemotaxis (Reviewed in Gerard, C., and Gerard, N. P. (1994) Annu. Rev. Immunol. 12, 775-808; Hugli, T. E. (1984) Springer Semin. Immunopathol. 7, 193-219; Bitter-Suermann, D. (1988) in The Complement System, Ed. by K. Rother & G. Till, Springer Verlag, Heidelberg 367-395).

Studies have also demonstrated the presence of a C3a receptor (C3a-R) on guinea pig platelets, rat mast cells, human neutrophils, eosinophils and platelets (Bitter-Suermann, D. (1988) in The Complement System, Ed. by K. Rother & G. Till, Springer Verlag, Heidelberg 367-395). A single class of high affinity C3a binding sites has been characterized in humans.

Complement activation has been implicated in the pathogenesis of inflammatory neurodegenerative disorders in the CNS and PNS, such as Alzheimer's disease, Huntington's disease, Pick's disease, and Gullian Barre syndrome. (See Campos-Torres et al.., (August 2000), Immunopharmacology, volume 49, Issues 1-2, page 48; see also, (Vogt, W. (1986) *Complement* 3, 177-188; Morgan, B. P. (1994) *European J. Clin. Investigation,* 24, 219-228; and Morgan et al., (1997), Immunopharmacology, 38, 43-50)

Abe et al. have shown that complement activation plays a significant role in allergic lung damage caused by repeated inhalation of antigen, a model ~~which is consistent with the etiology~~ of asthma. (See Abe et al., Immunopharmacology, Volume 49, Issues 1-2, page 26 (August 2000))

Additionally, Kirshfink reported that controlling activation of the complement system ~~can impact on the treatment or prevention of disease states such as~~ decreases the severity of sepsis,

adult respiratory distress syndrome, nephrites, graft rejection, myocardial ischemia/reperfusion injury, and intestinal ischemia/reperfusion injury. (See Kirshfink, M., (1997), Immunopharmacology, 38, 51-62; see also Lucchesi et al., (1997), Immunopharmacology, 38, 27-42)

Thus a large body of evidence indicates that activation of the complement system participates in both peripheral and central inflammatory diseases. In particular, the anaphylatoxins C3a and C5a are implicated as peptide messengers that propagate the inflammatory response driven by complement system activation. ~~Since chronic inflammatory responses usually lead to local tissue damage, and since many of the products of microglial activation are known neurotoxins,~~ bBlocking activation of the C3a receptor ~~this inflammatory response~~ with small molecule antagonists will likely be of ~~significant~~ therapeutic benefit in treating ~~these complement mediated inflammatory conditions.~~ a broad range of inflammatory diseases. ~~Thus there remains a need for discovering novel therapies for complement mediated inflammatory diseases.~~

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 are structural representations of certain compounds of the present invention.

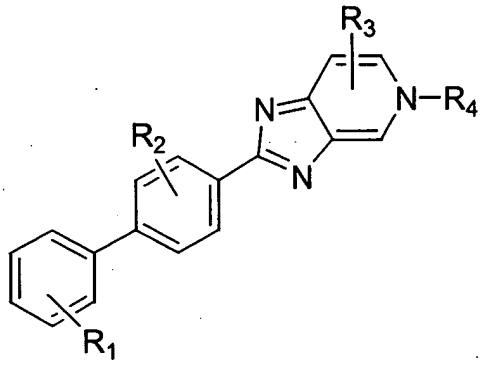
FIG. 2 are structural representations of other compounds of the present invention.

FIG. 3 are structural representations of further compounds of the present invention.

FIG. 4 is a diagrammatic representation of the complement cascade.

SUMMARY OF THE INVENTION

One aspect of the present invention are compounds represented by Formula I



(I)

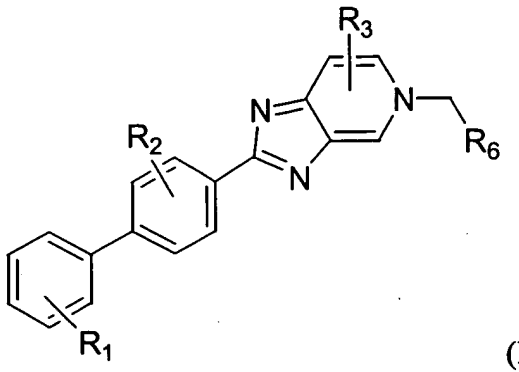
wherein,

R_1, R_2, R_3, and R_4,independently for each occurrence, represent one or more substituents selected from hydrogen, hydroxy, halo, amino, $C(=X)R_7$ (wherein X is NR_7, O or S), $-OC(=O)-R_7$, $-C(=O)O-R_7$, $-N(R_7)_2$, $-NR_7C(=O)-R_7$, $-C(=O)N(R_7)_2$, $-OC(=O)-N(R_7)_2$, $-NR_7-C(=O)-N(R_7)_2$, $-NR_7-C(NR_7)-N(R_7)_2$, $-P(O)_n-$ (wherein n is 0, 1, or 2), $-S(O)_n-$ (wherein n is 0, 1, or 2), $-S(O)_nN(R_7)_2$, (C_1-C_6)alkoxy-, (C_1-C_6)acyloxy-, (C_1-C_6)alkylamino-, $((C_1-C_6)alkyl)_2$amino-, (C_1-C_6)acylamino-, cyano, nitro, (un)substituted (C_1-C_6)alkyl-, (un)substituted (C_2-C_6)alkenyl-, (un)substituted (C_2-C_6)alkynyl-, (un)substituted (C_6-C_{10})aryl, (un)substituted (C_4-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl-, and (un)substituted (C_2-C_{10})heterocycloalkyl-;

or R_3 and R_4, taken together form a (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_3-C_{10})heterocycloalkyl, (un)substituted (C_5-C_{10})aryl, or (un)substituted (C_4-C_{10})heteroaryl; and

R_7, independently for each occurrence, represents one or more of H, (un)substituted (C_1-C_{10})alkyl, (un)substituted (C_2-C_{10})alkenyl , (un)substituted (C_2-C_{10})alkynyl, (C_6-C_{10})aryl, (un)substituted (C_1-C_{10})heteroalkyl, (un)substituted (C_5-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_2-C_{10})heterocycloalkyl.

Another aspect of the present invention is a compound represented by Formula II



(II)

wherein,

R_1, R_2, R_3, and R_6,independently for each occurrence, represent one or more substituents selected from hydrogen, hydroxy, halo, amino, $C(=X)R_7$ (wherein X is NR_7, O or S), $-OC(=O)-R_7$, $-C(=O)O-R_7$, $-N(R_7)_2$, $-NR_7C(=O)-R_7$, $-C(=O)N(R_7)_2$, $-OC(=O)-N(R_7)_2$, $-NR_7-C(=O)-N(R_7)_2$, $-NR_7-C(NR_7)-N(R_7)_2$, $-P(O)_n-$ (wherein n is 0, 1, or 2), $-S(O)_n-$ (wherein n is 0, 1, or 2), $-S(O)_nN(R_7)_2$, (C_1-C_6)alkoxy-, (C_1-C_6)acyloxy-, (C_1-C_6)alkylamino-, $((C_1-C_6)alkyl)_2$amino-, (C_1-C_6)acylamino-, cyano, nitro, (un)substituted (C_1-C_6)alkyl-, (un)substituted (C_2-C_6)alkenyl-, (un)substituted (C_2-C_6)alkynyl-, (un)substituted (C_6-C_{10})aryl, (un)substituted (C_4-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl-, and (un)substituted (C_2-C_{10})heterocycloalkyl-;

or R_3 and R_4, taken together form a (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_3-C_{10})heterocycloalkyl, (un)substituted (C_5-C_{10})aryl, or (un)substituted (C_4-C_{10})heteroaryl; and

R_7, independently for each occurrence, represents one or more of H, (un)substituted (C_1-C_{10})alkyl, (un)substituted (C_2-C_{10})alkenyl , (un)substituted (C_2-C_{10})alkynyl, (C_6-C_{10})aryl, (un)substituted (C_1-C_{10})heteroalkyl, (un)substituted (C_5-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl, and (un)substituted (C_2-C_{10})heterocycloalkyl.

Another aspect of the present invention is a pharmaceutical composition comprising a compound(s) of the present invention, pharmaceutically acceptable addition salts thereof, or substantially enriched enantiomeric forms thereof and a pharmaceutically acceptable carrier. In certain embodiments these compositions may be formulated in unit dosage forms. In certain other compositions, these compositions may be formulated in a sustained release formulation comprising a biocompatible polymer.

Another aspect of the present invention is a method for preventing excessive complement activation in a patient comprising administering to said patient, a therapeutically effective amount of a compound(s) of the present invention.

Another aspect of the present invention is a method for treating or preventing complement-mediated tissue damage in a patient comprising administering to said patient, a therapeutically effective amount of a compound(s) of the present invention.

Another aspect of the present invention is a method for treating diseases characterized by chronic complement activation comprising administering to a patient a therapeutically effective amount of a compound(s) of the present invention.

Another aspect of the present invention is a method for treating Alzheimer's disease comprising administering to a patient a therapeutically effective amount of a compound(s) of the present invention.

Another aspect of the present invention is a method for treating Huntington's disease comprising administering to a patient a therapeutically effective amount of a compound(s) of the present invention.

Another aspect of the present invention is a method for treating Pick's disease comprising administering to a patient a therapeutically effective amount of a compound(s) of the present invention.

Another aspect of the present invention is a method for treating asthma comprising administering to a patient a therapeutically effective amount of a compound(s) of the present invention.

Another aspect of the present invention is a method for treating or preventing a medical condition selected from the group consisting of sepsis, adult respiratory distress syndrome, nephrites, graft rejection, myocardial ischemia/reperfusion injury, and intestinal ischemia/reperfusion injury, comprising administering to a patient a therapeutically effective amount of a compound(s) of the present invention.

Another aspect of the present invention is a method for antagonizing the C3a receptor in a patient by administering an effective amount of a compound(s) of the present invention.

Another aspect of the present invention is a method for antagonizing the C5a receptor in a patient by administering an effective amount of a compound(s) of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

In connection with the practice of this invention, the following definitions will generally apply.

"Antagonist" as used herein is meant to refer to an agent that down-regulates (e.g., suppresses or inhibits) the bioactivity of a protein of interest, or an agent that inhibits/suppresses or reduces (e.g., destabilizes or decreases) interaction among polypeptides or other molecules (e.g., steroids, hormones, nucleic acids, etc.). An antagonist can also be a compound that down-regulates the expression of a gene of interest or which reduces the amount of the wild-type protein present. An antagonist can also be a protein or small molecule which decreases or inhibits the interaction of a polypeptide of interest with another molecule, e.g., a target peptide or nucleic acid.

The terms "biocompatible polymer" and "biocompatibility" when used in relation to the pharmaceutical compositions of the present invention are art-recognized. For example, biocompatible polymers include polymers that are neither themselves toxic to the host (e.g., an animal or human), nor degrade (if the polymer degrades) at a rate that produces monomeric or oligomeric subunits or other byproducts at toxic concentrations in the host.

An 'effective amount' of a subject compound, with respect to the subject method of treatment, refers to an amount of the therapeutic in a preparation which, when applied as part of a desired dosage regimen provides a benefit according to clinically acceptable standards for the treatment or prophylaxis of a particular disorder.

In certain embodiments, the compounds of the present invention may have a high affinity for the C3a receptor. In certain embodiments, the compounds of the present invention may have a high affinity for the C5a receptor. The term "high affinity" as used herein means a strong binding affinity between molecules with a dissociation constant K_D of no greater than 1 μM. In a preferred case, the K_D is less than 100 nM, 10 nM, 1 nM, 100 pM, or even 10 pM or less.

A "patient" or "subject" to be treated by the subject method can mean either a human or non-human subject.

The term "prophylactic or therapeutic" treatment is art-recognized and includes the administration to the host of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the

host animal) then the treatment is prophylactic, i.e., it protects the host against developing the unwanted condition, whereas if it is administered after manifestation of the unwanted condition, the treatment is therapeutic, (i.e., it is intended to diminish, ameliorate, or stabilize the existing unwanted condition or side effects thereof).

The compounds of the present invention may be administered to a patient in an amount that is therapeutically effective. A "therapeutically effective amount" of a compound, with respect to a method of treatment, refers to an amount of the compound(s) in a preparation which, when administered as part of a desired dosage regimen (to a mammal, preferably a human) alleviates a symptom, ameliorates a condition, or slows the onset of disease conditions according to clinically acceptable standards for the disorder or condition to be treated or the cosmetic purpose, e.g., at a reasonable benefit/risk ratio applicable to any medical treatment.

A "therapeutically effective daily dosage" of a compound, with respect to a method of treatment, refers to an amount of the compound(s) in a preparation which, when administered as part of a desired daily dosage regimen (to a mammal, preferably a human) alleviates a symptom, ameliorates a condition, or slows the onset of disease conditions according to clinically acceptable standards for the disorder or condition to be treated or the cosmetic purpose, e.g., at a reasonable benefit/risk ratio applicable to any medical treatment.

The term "treating", as used herein, refers to reversing, alleviating, inhibiting the progress of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition. The term "treatment", as used herein, refers to the act of treating, as "treating" is defined immediately above.

The term "alkyl" refers to a saturated branched or unbranched carbon chain radical having the number of carbon atoms specified, or up to 30 carbon atoms if no specification is made. For example, an alkyl of 1 to 8 carbon atoms refers to radicals such as methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, and octyl, and those radicals which are positional isomers of these radicals. An alkyl of 10 to 30 carbon atoms includes decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, eicosyl, heneicosyl, docosyl, tricosyl and tetracosyl. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C_1-C_{30} for straight chains, C_3-C_{30} for branched chains), and more preferably 20 or fewer, and most preferably 10 or fewer carbon atoms in its backbone. Likewise, preferred cycloalkyls have from 3-10 carbon atoms in their

ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure. Similarly, the terms "alkenyl" and "alknyl" define hydrocarbon radicals having straight, branched or cyclic moities wherein at least one double bond, or at least one triple bond, respectively, is present. Such definitions also apply when the alkyl, alkenyl or alkynyl group is present within another group, such as alkoxy or alkylamine.

An "aryl" group as used herein, unless otherwise indicated, includes an organic radical derived from a monocyclic or bicylic (C_6-C_{10}) aromatic hydrocarbon compound by removal of a hydrogen radical from a ring carbon of the aryl compound. An aryl group is optionally substituted by one or more substituents mentioned above wherein, unless otherwise indicated, the selection of each optional substituent is independent of the selection of any other optional substituents, and preferably the number of optional substituents is between 0 and 3, more preferably between 0 and 2. Representative aryl groups include phenyl and naphthyl groups.

A "cycloalkyl" group as used herein, unless otherwise indicated, includes an organic radical derived from a monocyclic (C_3-C_{10})cycloalkyl compound, by removal of a hydrogen radical from a ring carbon of the cycloalkyl compound. A cycloalkyl group is optionally substituted by one or more substituents wherein, unless otherwise indicated, the selection of each optional substituent is independent of the selection of any other optional substituents, and preferably the number of optional substituents is between 0 and 3, more preferably between 0 and 2. Representative cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, 1 ,3-cyclobutadienyl, 1 ,3-cyclopentadienyl, 1 ,3-cyclohexadienyl, 1,4-cyclohexadienyl, I ,3-cycloheptadienyl, 1,4-cycloheptadienyl, I ,3,5-cycloheptatrienyl, bicyclo[3.2.l]octane, bicyclo[2.2.1] heptane, and the norborn-2-ene unsaturated form thereof. Thus, the term cycloalkyl also includes cycloalkenyl groups having one or two double bonds.

A "heteroaryl" group as used herein, unless otherwise indicated, includes an organic radical derived from a monocyclic or bicyclic (C_4-C_{10}) aromatic heterocyclic compound by removal of a hydrogen radical from a ring atom of the heteroaryl compound, said ring atom being uncharged in said compound. A heteroaryl group is optionally substituted by one or more substituents wherein, unless otherwise indicated, the selection of each optional substituent is independent of the selection of any other optional substituent, and preferably the number of optional substituents is between 0 and 3, more preferably between 0 and 2. Representative

heteroaryl groups include furyl, thienyl, thiazolyl, pyrazolyl, isothiazolyl, oxazolyl, isoxazolyl, pyrrolyl, triazolyl, tetrazolyl, imidazolyl, 1,3,5oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,3-oxadiazolyl, 1,3-thiadiazolyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, pyridyl, pyrimidyl, pyrazinyl, pyridazinyl, 1,2,4-triazinyl, 1,2,3-triazinyl, 1,3,5triazinyl, pyrazolo[3,4-blpyridinyl, cinnolinyl, pteridinyl, purinyl, 6,7-dihydro-5H-[llpyrindinyl, benzo[b]thiophenyl, 5, 6, 7, 8-tetrahydro-quinolin-3-yl, benzoxazolyl, benzothiazolyl, benzisothiazolyl, benzisoxazolyl, benzimidazolyl, thianaphthenyl, isothianaphthenyl, benzofuranyl, isobenzofuranyl, isoindolyl, indolyl, indolizinyl, indazolyl, isoquinolyl, quinolyl, phthalazinyl, quinoxalinyl, quinazolinyl, and benzoxazinyl; and the like.

A "heterocycloalkyl" group as used herein, unless otherwise indicated, includes an organic radical derived from a monocyclic (C_3-C_{10}) heterocycloalkyl compound by removal of a hydrogen radical from a ring atom of the heterocycloalkyl compound. A heterocycloalkyl group is optionally substituted by one or more substituents wherein, unless otherwise indicated, the selection of each optional substituent is independent of the selection of any other optional substituents, and preferably the number of optional substituents is between 0 and 3, more preferably between 0 and 2. Representative heterocycloalkyl groups include pyrrolidinyl, tetrahydrofuranyl, dihydrofuranyl, tetrahydropyranyl, pyranyl, thiopyranyl, aziridinyl, oxiranyl, methylenedioxyl, chromenyl, isoxazolidinyl, 1,3-oxazolidin-3-yl, isothiazolidinyl, 1,3-thiazolidin-3-yl, 1,2-pyrazolidin-2-yl, 1,3-pyrazolidin-l-yl, piperidinyl, thiomorpholinyl, 1,2-tetrahydrothiazin-2-yl, 1 ,3-tetrahydrothiazin-3-yl, tetrahydrothiadiazinyl, morpholinyl, 1,2-tetrahydrodiazin-2-yl, 1,3-tetrahydrodiazin-l-yl, tetrahydroazepinyl, piperazinyl, and chromanyl.

In connection with the terms "alkyl" group, "alkenyl" group, "alkynyl" group, "heteroalkyl" group, "aryl" group, "heteroaryl" group, "cycloalkyl" group and "heterocycloalkyl" group, as herein defined, the term "optionally substituted" means that one or more chemically and pharmaceutically acceptable functional groups may be bonded thereto. Such functional groups contribute properties useful to the production, storage, or use of the inventive compounds as pharmaceuticals, or at least does not substantially negate their pharmacological activity. Such suitable substituents may be determined by those skilled in the art. Illustrative examples of suitable substituents include, but are not limited to, hydrogen, hydroxy, halo, amino, $C(=X)R_7$ (wherein X is NR_7, O or S), -OC(=O)-, -C(=O)O, -NR_7-, -NR_7C(=O)-, -C(=O)NR_7-, -OC(=O)-NR_7, -NR_7-C(=O)-NR_7-, -NR_7-C(NR_7)-NR_7-, -$P(O)_n$- (wherein n is 0, 1, or 2), -$S(O)_n$- (wherein n is 0, 1, or 2), -$S(O)_n$$NHR_7$ (wherein n is 0, 1, or 2),

$NR_7S(O)_n$- (wherein n is 0, 1, or 2),and -$B(OR_7)$-, (C_1-C_6)alkoxy-, (C_1-C_6)acyloxy-, (C_1-C_6)alkylamino-, ((C_1-C_6)alkyl)$_2$amino-, (C_1-C_6)acylamino-, cyano, nitro, (C_1-C_6)alkyl-, (C_1-C_6)alkenyl-, (C_1-C_6)alkynyl-, (C_1-C_6)acylamino-, cyano(C_1-C_6)alkyl-, trifluoromethyl(C_1-C_6)alkyl-, nitro(C_1-C_6)alkyl-, (C_1-C_6)alkyl(difluoromethylene)(C_1-C_6)alkyl-, (C_1-C_6)acylamino(C_1-C_6)alkyl-, (C_1-C_6)alkoxy(C_1-C_6)acylamino-, amino(C_1-C_6)acyl-, amino(C_1-C_6)acyl(C_1-C_6)alkyl-, (C_1-C_6)alkylamino(C_1-C_6)acyl-, ((C_1-C_6)alkyl)$_2$amino(C_1-C_6)acyl-, (C_3-C_{10})cycloalkyl(C_1-C_6)alkyl-, (C_1-C_6)acyloxy((C_1-C_6)alkyl-, (C_1-C_6)alkoxy(C_1-C_6)alkyl-, (C_1-C_6)acylamino(C_1-C_6)alkyl-, (C_5-C_{10})aryl(C_1-C_6)alkoxy(C_1-C_6)alkyl-, (C_4-C_{10})heteroaryl(C_1-C_6)alkoxy(C_1-C_6)alkyl-, (C_1-C_6)alkylthio(C_1-C_6)alkyl-, (C_6-C_{10})arylthio(C_1-C_6)alkyl-, (C_1-C_6)alkylsulfinyl(C_1-C_6)alkyl-, (C_6-C_{10})arylsulfinyl(C_1-C_6)alkyl-, (C_1-C_6)alkylsulfonyl(C_1-C_6)alkyl-, (C_6-C_{10})arylsulfonyl(C_1-C_6)alkyl-, amino(C_1-C_6)alkyl-, (C_1-C_6)alkylamino(C_1-C_6)alkyl-, (C_1-C_6)alkoxy(C_1-C_6)acyl-, (C_1-C_6)alkylamino(C_1-C_6)acyl-, ((C_1-C_6)alkyl)$_2$amino(C_1-C_6)acyl-, (C_6-C_{10})aryl-, (C_4-C_{10})heteroaryl-, (C_6-C_{10})aryl(C_1-C_6)alkyl-, (C_4-C_{10})heteroaryl(C_1-C_6)alkyl-, (C_6-C_{10})aryl(C_5-C_{10})aryl-, , (C_3-C_{10})cycloalkyl-, (C_3-C_{10})cycloalkyl(C_1-C_6)alkyl-, (C_2-C_{10})heterocycloalkyl-, (C_2-C_{10})heterocycloalkyl(C_1-C_6)alkyl-, hydroxy(C_1-C_6)alkyl-; and R_7, independently for each occurrence, represents one or more occurrences of H, (un)substituted (C_1-C_{10})alkyl, (un)substituted (C_2-C_{10})alkenyl , (un)substituted (C_2-C_{10})alkynyl, (C_6-C_{10})aryl, (un)substituted (C_1-C_{10})heteroalkyl, (un)substituted (C_5-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_2-C_{10})heterocycloalkyl.

Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including cis- and trans-isomers, *(R)*- and *(S)*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

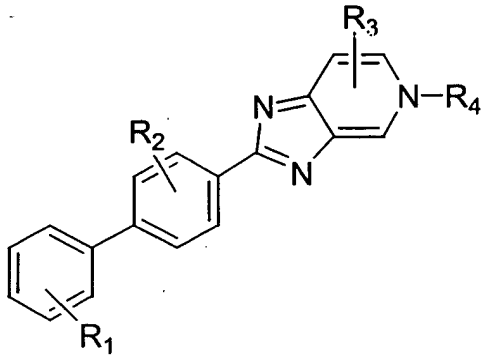
If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivatization with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts may be

formed with an appropriate optically active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

Contemplated equivalents of the compounds described above include compounds which otherwise correspond thereto, and which have the same general properties thereof, wherein one or more simple variations of substituents are made which do not adversely affect the efficacy of the compound. In general, the compounds of the present invention may be prepared by the methods illustrated in the general reaction schemes as, for example, described below, or by modifications thereof, using readily available starting materials, reagents and conventional synthesis procedures. In these reactions, it is also possible to make use of variants which are in themselves known, but are not mentioned here.

For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 67th Ed., 1986-87, inside cover. Also for purposes of this invention, the term "hydrocarbon" is contemplated to include all permissible compounds having at least one hydrogen and one carbon atom. In a broad aspect, the permissible hydrocarbons include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic organic compounds which can be substituted or unsubstituted.

One aspect of the present invention is a compound represented by Formula I



(I)

wherein,

R_1, R_2, R_3, and R_4,independently for each occurrence, represent one or more substituents selected from hydrogen, hydroxy, halo, amino, $C(=X)R_7$ (wherein X is NR_7, O or S),

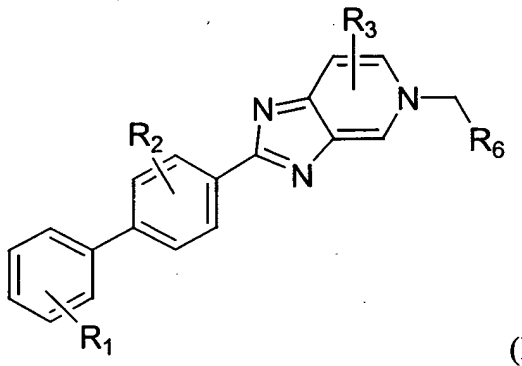
-OC(=O)-R_7, -C(=O)O-R_7, -N(R_7)$_2$, -NR_7C(=O)-R_7, -C(=O)N(R_7)$_2$, -OC(=O)-N(R_7)$_2$, -NR_7-C(=O)-N(R_7)$_2$, -NR_7-C(NR_7)-N(R_7)$_2$, -P(O)$_n$- (wherein n is 0, 1, or 2), -S(O)$_n$- (wherein n is 0, 1, or 2), -S(O)$_n$N(R_7)$_2$, (C_1-C_6)alkoxy-, (C_1-C_6)acyloxy-, (C_1-C_6)alkylamino-, ((C_1-C_6)alkyl)$_2$amino-, (C_1-C_6)acylamino-, cyano, nitro, (un)substituted (C_1-C_6)alkyl-, (un)substituted (C_2-C_6)alkenyl-, (un)substituted (C_2-C_6)alkynyl-, (un)substituted (C_6-C_{10})aryl, (un)substituted (C_4-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl-, (un)substituted (C_2-C_{10})heterocycloalkyl-;

or R_3 and R_4, taken together form a (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_3-C_{10})heterocycloalkyl, (un)substituted (C_5-C_{10})aryl, or (un)substituted (C_4-C_{10})heteroaryl; and

R_7, independently for each occurrence, represents one or more of H, (un)substituted (C_1-C_{10})alkyl, (un)substituted (C_2-C_{10})alkenyl , (un)substituted (C_2-C_{10})alkynyl, (C_6-C_{10})aryl, (un)substituted (C_1-C_{10})heteroalkyl, (un)substituted (C_5-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_2-C_{10})heterocycloalkyl.

In certain embodiments, R_1, R_2, and R_3, independently for each occurrence, are selected from one or more of a hydrogen, a halogen, a (C_1-C_6)alkyl-, and a (C_1-C_6)alkoxyl-.

Another aspect of the present invention is a compound represented by Formula II,



(II)

wherein,

R_1, R_2, R_3, and R_6,independently for each occurrence, represent one or more substituents selected from hydrogen, hydroxy, halo, amino, C(=X)R_7 (wherein X is NR_7, O or S), -OC(=O)-R_7, -C(=O)O-R_7, -N(R_7)$_2$, -NR_7C(=O)-R_7, -C(=O)N(R_7)$_2$, -OC(=O)-N(R_7)$_2$, -NR_7-C(=O)-N(R_7)$_2$, -NR_7-C(NR_7)-N(R_7)$_2$, -P(O)$_n$- (wherein n is 0, 1, or 2), -S(O)$_n$- (wherein n is 0, 1, or 2), -S(O)$_n$N(R_7)$_2$, (C_1-C_6)alkoxy-, (C_1-C_6)acyloxy-, (C_1-C_6)alkylamino-, ((C_1-C_6)alkyl)$_2$amino-, (C_1-C_6)acylamino-, cyano, nitro, (un)substituted (C_1-C_6)alkyl-,

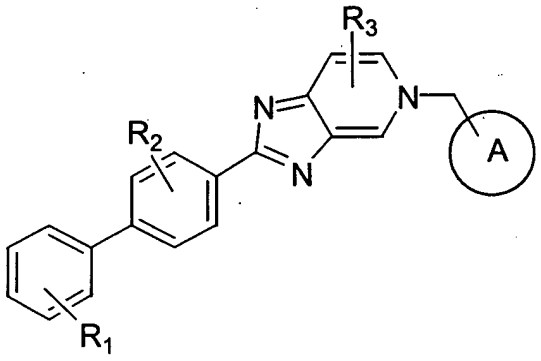
(un)substituted (C_2-C_6)alkenyl-, (un)substituted (C_2-C_6)alkynyl-, (un)substituted (C_6-C_{10})aryl, (un)substituted (C_4-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl-, (un)substituted (C_2-C_{10})heterocycloalkyl-;

or R_3 and R_4, taken together form a (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_3-C_{10})heterocycloalkyl, (un)substituted (C_5-C_{10})aryl, or (un)substituted (C_4-C_{10})heteroaryl; and

R_7, independently for each occurrence, represents one or more of H, (un)substituted (C_1-C_{10})alkyl, (un)substituted (C_2-C_{10})alkenyl , (un)substituted (C_2-C_{10})alkynyl, (C_6-C_{10})aryl, (un)substituted (C_1-C_{10})heteroalkyl, (un)substituted (C_5-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_2-C_{10})heterocycloalkyl.

In certain preferred embodiments, R_6 is selected from a cyano, (C_1-C_6)alkyl, (C_2-C_{10})alkenyl, and (C_2-C_{10})alkynyl.

Another aspect of the present invention is a compound represented by Formula III:



(III)

wherein,

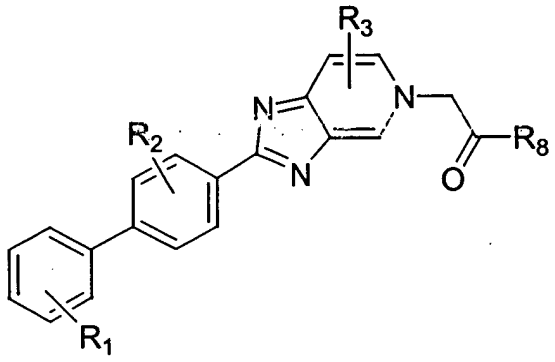
A is selected from an (un)substituted (C_6-C_{10})aryl, or (un)substituted (C_4-C_{10})heteroaryl;

R_1, R_2, and R_3 independently for each occurrence, represent one or more substituents selected from hydrogen, hydroxy, halo, amino, C(=X)R_7 (wherein X is NR_7, O or S), -OC(=O)-R_7, -C(=O)O-R_7, -N(R_7)$_2$, -NR_7C(=O)-R_7, -C(=O)N(R_7)$_2$, -OC(=O)-N(R_7)$_2$, -NR_7-C(=O)-N(R_7)$_2$, -NR_7-C(NR_7)-N(R_7)$_2$, -P(O)$_n$- (wherein n is 0, 1, or 2), -S(O)$_n$- (wherein n is 0, 1, or 2), -S(O)$_n$N(R_7)$_2$, (C_1-C_6)alkoxy-, (C_1-C_6)acyloxy-, (C_1-C_6)alkylamino-, ((C_1-C_6)alkyl)$_2$amino-, (C_1-C_6)acylamino-, cyano, nitro, (un)substituted (C_1-C_6)alkyl-, (un)substituted (C_2-C_6)alkenyl-, (un)substituted (C_2-C_6)alkynyl-, (un)substituted (C_6-C_{10})aryl, (un)substituted (C_4-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl-, (un)substituted (C_2-C_{10})heterocycloalkyl-;

or R_3 and R_4, taken together form a (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_3-C_{10})heterocycloalkyl, (un)substituted (C_6-C_{10})aryl, or (un)substituted (C_4-C_{10})heteroaryl; and

R_7, independently for each occurrence, represents one or more of H, (un)substituted (C_1-C_{10})alkyl, (un)substituted (C_2-C_{10})alkenyl , (un)substituted (C_2-C_{10})alkynyl, (C_6-C_{10})aryl, (un)substituted (C_1-C_{10})heteroalkyl, (un)substituted (C_5-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_2-C_{10})heterocycloalkyl.

Another aspect of the present invention is a compound represented by Formula IV:



(IV)

wherein,

R_1, R_2, and R_3, independently for each occurrence, represent one or more substitutents selected from hydrogen, halogen, and (C_1-C_6)alkyl-, (C_1-C_6)aryl-;

R_8 independently for each occurrence, represents one or more substituents selected from hydrogen, hydroxy, halo, amino, C(=X)R_7 (wherein X is NR_7, O or S), -OC(=O)-R_7, -C(=O)O-R_7, -N(R_7)$_2$, -NR_7C(=O)-R_7, -C(=O)N(R_7)$_2$, -OC(=O)-N(R_7)$_2$, -NR_7-C(=O)-N(R_7)$_2$, -NR_7-C(NR_7)-N(R_7)$_2$, -P(O)$_n$- (wherein n is 0, 1, or 2), -S(O)$_n$- (wherein n is 0, 1, or 2), -S(O)$_n$N(R_7)$_2$, (C_1-C_6)alkoxy-, (C_1-C_6)acyloxy-, (C_1-C_6)alkylamino-, ((C_1-C_6)alkyl)$_2$amino-, (C_1-C_6)acylamino-, cyano, nitro, (un)substituted (C_1-C_6)alkyl-, (un)substituted (C_2-C_6)alkenyl-, (un)substituted (C_2-C_6)alkynyl-, (un)substituted (C_6-C_{10})aryl, (un)substituted (C_4-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl-, (un)substituted (C_2-C_{10})heterocycloalkyl-;

or R_3 and R_4, taken together form a (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_3-C_{10})heterocycloalkyl, (un)substituted (C_6-C_{10})aryl, or (un)substituted (C_4-C_{10})heteroaryl; and

R_7, independently for each occurrence, represents one or more of H, (un)substituted (C_1-C_{10})alkyl, (un)substituted (C_2-C_{10})alkenyl , (un)substituted (C_2-C_{10})alkynyl, (C_6-C_{10})aryl,

(un)substituted (C_1-C_{10})heteroalkyl, (un)substituted (C_5-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_2-C_{10})heterocycloalkyl.

In certain embodiments, the compound may be selected from 2-(3',4'-dimethyl-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-methyl-5H-imidazo[4,5-c]pyridine; [2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetic acid methyl ester; [2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetonitrile; 5-allyl-2-(3',4'-dimethyl-biphenyl-4-yl)-5H-imidazo[4,5-c]pyridine; 1-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-propan-2-one; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-prop-2-ynyl-5H-imidazo[4,5-c]pyridine; 4-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-3-ethoxy-but-2-enoic acid ethyl ester; 5-benzyl-2-(3',4'-dimethyl-biphenyl-4-yl)-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-pyridin-3-ylmethyl-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-pyridin-4-ylmethyl-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-pyridin-2-ylmethyl-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-[1-(toluene-4-sulfonyl)-1H-imidazol-2-ylmethyl]-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-[1-(toluene-4-sulfonyl)-1H-imidazol-4-ylmethyl]-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(5-methyl-[1,3,4]oxadiazol-2-ylmethyl)-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-thiazol-2-ylmethyl-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-[2-(1H-imidazol-4-yl)-ethyl]-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(3-methyl-3H-imidazol-4-ylmethyl)-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(1-methyl-1H-imidazol-4-ylmethyl)-5H-imidazo[4,5c]pyridine; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2-methyl-1H-imidazol-4-yl)-ethanone; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone; 1-(2-Amino-4-methyl-thiazol-5-yl)-2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-ethanone; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-[2-(2H-pyrazol-3-yl)-ethyl]-5H-imidazo[4,5-c]pyridine; 2-[2-(4'-methoxy-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone hydrochloride; 2-{2-[4-(1-methyl-5-trifluoromethyl-1H-pyrazol-3-yl)-phenyl]-imidazo[4,5-c]pyridin-5-yl}-1-(2H-pyrazol-3-yl)-ethanone maleate; 2-[2-(4'-fluoro-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone hydrochloride; 2-[2-(3'-methyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone; 2-[2-(3'-Chloro-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone hydrochloride; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(2H-pyrazol-3-

(un)substituted (C_1-C_{10})heteroalkyl, (un)substituted (C_5-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_2-C_{10})heterocycloalkyl.

In certain embodiments, the compound may be selected from 2-(3',4'-dimethyl-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-methyl-5H-imidazo[4,5-c]pyridine; [2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetic acid methyl ester; [2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetonitrile; 5-allyl-2-(3',4'-dimethyl-biphenyl-4-yl)-5H-imidazo[4,5-c]pyridine; 1-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-propan-2-one; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-prop-2-ynyl-5H-imidazo[4,5-c]pyridine; 4-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-3-ethoxy-but-2-enoic acid ethyl ester; 5-benzyl-2-(3',4'-dimethyl-biphenyl-4-yl)-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-pyridin-3-ylmethyl-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-pyridin-4-ylmethyl-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-pyridin-2-ylmethyl-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-[1-(toluene-4-sulfonyl)-1H-imidazol-2-ylmethyl]-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-[1-(toluene-4-sulfonyl)-1H-imidazol-4-ylmethyl]-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(5-methyl-[1,3,4]oxadiazol-2-ylmethyl)-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-thiazol-2-ylmethyl-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-[2-(1H-imidazol-4-yl)-ethyl]-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(3-methyl-3H-imidazol-4-ylmethyl)-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(1-methyl-1H-imidazol-4-ylmethyl)-5H-imidazo[4,5c]pyridine; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2-methyl-1H-imidazol-4-yl)-ethanone; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone; 1-(2-Amino-4-methyl-thiazol-5-yl)-2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-ethanone; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-[2-(2H-pyrazol-3-yl)-ethyl]-5H-imidazo[4,5-c]pyridine; 2-[2-(4'-methoxy-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone hydrochloride; 2-{2-[4-(1-methyl-5-trifluoromethyl-1H-pyrazol-3-yl)-phenyl]-imidazo[4,5-c]pyridin-5-yl}-1-(2H-pyrazol-3-yl)-ethanone maleate; 2-[2-(4'-fluoro-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone hydrochloride; 2-[2-(3'-methyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone; 2-[2-(3'-Chloro-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone hydrochloride; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(2H-pyrazol-3-

ylmethyl)-5H-imidazo[4,5-c]pyridine hydrochloride; 2-(3'-chloro-biphenyl-4-yl)-5-(2H-pyrazol-3-ylmethyl)-5H-imidazo[4,5-c]pyridine hydrochloride; 2-(3'-methyl-biphenyl-4-yl)-5-(2H-pyrazol-3-ylmethyl)-5H-imidazo[4,5-c]pyridine hydrochloride; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-ethanol; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-ethylamine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(1H-tetrazol-5-ylmethyl)-5H-imidazo[4,5-c]pyridine1-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-propan-2-ol; 1-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-propan-2-one O-methyl-oxime; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-N,N-dimethyl-acetamide; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetamide; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-N-methyl-acetamide; 5-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-1,2-dihydro-pyrazol-3-one; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(1H-imidazol-2-ylmethyl)-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(1H-imidazol-4-ylmethyl)-5H-imidazo[4,5-c]pyridine; 5-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-2,4-dihydro-[1,2,4]triazol-3-one; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(1H-[1,2,4]triazol-3-ylmethyl)-5H-imidazo[4,5c]pyridine; 5-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-3H-[1,3,4]oxadiazol-2-one; 5-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-[1,3,4]oxadiazol-2-ylamine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(2-methyl-1H-imidazol-4-ylmethyl)-5H-imidazo[4,5c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(1H-pyrazol-4-ylmethyl)-5H-imidazo[4,5-c]pyridine; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-{2-methyl-1-[2-(2-methyl-1H-imidazol-4-yl)-2-oxo-ethyl]-1H-imidazol-4-yl}-ethanone: 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2-methyl-1H-imidazol-4-yl)-ethanol; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanol; 2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-pyrazin-2-yl-ethanol; 2-(4-bromo-phenyl)-3H-imidazo[4,5-c]pyridine; 2-[4-(1-methyl-5-trifluoromethyl-1H-pyrazol-3-yl)-phenyl]-3H-imidazo[4,5-c]pyridine hydrochloride; 2-(4'-methoxy-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine; 2-(4'-fluoro-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine; 2-(3'-methyl-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine; and 2-(3'-chloro-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine.

The compounds of Formula (I), (II), and some of the intermediates in the present invention may contain one or more asymmetric carbons. Pure stereochemically isomeric forms of said compounds and said intermediates can be obtained by the application of art-known

procedures. For example, diastereoisomers can be separated by physical methods such as selective crystallization or chromatographic techniques, e.g. counter current distribution, liquid chromatography and the like methods. Enantiomers can be obtained from racemic mixtures by first converting said racemic mixtures with suitable resolving agents such as, for example, chiral acids, to mixtures of diastereomeric salts or compounds; then physically separating said mixtures of diastereomeric salts or compounds by, for example, selective crystallization or chromatographic techniques, e.g. liquid chromatography and the like methods; and finally converting said separated diastereomeric salts or compounds into the corresponding enantiomers. Pure stereochemically isomeric forms of the compounds of Formula (I) or (II) may also be obtained from the pure stereochemical forms of the appropriate intermediates and starting materials, provided that the intervening reactions occur stereospecifically. The pure and mixed stereochemically isomeric forms of the compounds of Formula (I) or (II) are intended to be embraced within the scope of the present invention.

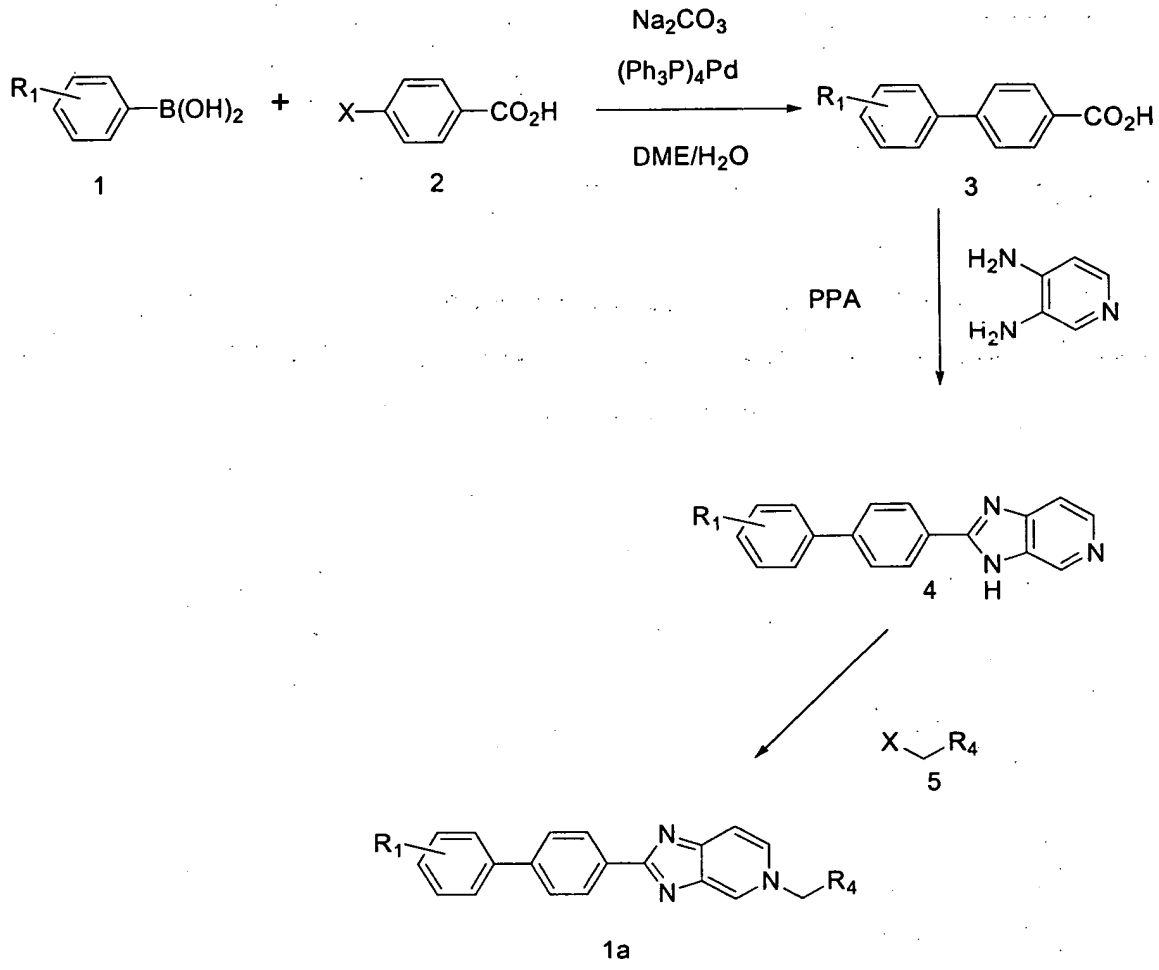
Another aspect of the present invention is a pharmaceutical composition comprising a compound(s) of the present invention, or pharmaceutically acceptable addition salts thereof, and a pharmaceutically acceptable carrier. In certain embodiments these compositions may be formulated in unit dosage forms.

The subject invention also includes isotopically-labelled compounds, which are identical to those recited in Formula (I) or Formula (II), but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{19}F, and ^{36}Cl, respectively. Compounds of the present invention, prodrugs thereof, and pharmaceutically acceptable salts of said compounds or of said prodrugs which contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of this invention. Certain isotopically-labelled compounds of the present invention, for example those into which radioactive isotopes such as 3H and 14C are incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, i.e., ^{3}H, and ^{14}C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium, i.e., ^{2}H, can afford certain therapeutic advantages resulting from greater metabolic

stability, for example increased in vivo half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labelled compounds of Formula (I) or Formula (II) of this invention and prodrugs thereof can generally be prepared by carrying out the procedures disclosed in the Schemes and/or in the Examples and Preparations below, by substituting a readily available isotopically labelled reagent for a non-isotopically labelled reagent.

One aspect of the present invention is a method of synthesizing the C3a antagonists described herein. The following reaction scheme is intended to illustrate the preparation of the antagonists of the present invention. It will be appreciated that certain groups represented by letters ("R" groups and the like) in the reaction scheme do not always correspond with similarly defined component groups of the Formula (I) or Formula (II) compounds themselves, since certain functionalities of the reactants can be modified when the resulting products are formed.

Scheme 1


Na2CO3
(Ph3P)4Pd
DME/H2O
R1
B(OH)2
X
CO2H
1
2
3
PPA
H2N
H2N
N
4
N
H
X
R4
5
1a

Scheme 1 above illustrates a general method suitable for the preparation of compounds of formula **1a**. Synthesis of the biphenyl carboxylic acid **3** involves treatment of phenylboronic acid **1** with benzoic acid **2** substituted with chloride, bromide, iodide or triflate, where the bromide is preferred, in the presence of a phosphorylated palladium species, where $(Ph_3P)_4Pd$ is preferred, or a mixture of palladium species, such as palladium acetate or tris(dibenzylidineacetone)dipalladium and a triarylphosphine such as triphenylphosphine, tri(o-tolyl)phosphine or a trialkylphosphine such as tri t-butylphosphine, a base such as an alkali metal carbonate, an alkali metal phosphate, an alkali metal hydroxide or alkali metal fluoride, where sodium carbonate is preferred, in a protic or non-protic solvent such as benzene, toluene, ethanol, tetrahydrofuran, dimethylformamide, water or preferably 1,2-dimethoxyethane, at a temperature from about 40°C to 160°C, where 80 to 120°C is preferred. Conversion of **3** into **4** can be achieved by treatment of **3** with 3,4-diaminopyridine in the presence of an acidic dehydrating agent such as phosphorus oxychloride or preferably polyphosphoric acid at temperatures ranging from 140 to 260°C where 200°C is preferred. Alkylation of **4** to give **1a** can be accomplished by deprotonation of **4** with a metal hydride base such as potassium hydride or sodium hydride, where sodium hydride is preferred, in a polar aprotic solvent such as dimethylformamide, tetrahydrofuran or preferably dimethylsulfoxide followed by treatment with an alkylating agent **5** at a temperature of 15-50°C where ambient temperature is preferred. The R4 group in compounds **1a** may be then further modified into other R4 groups by methods that will be immediately obvious to those skilled in the art.

Another aspect of the present invention is a pharmaceutical composition comprising substantially enriched enantiomeric forms of the compound(s) of the present invention, or pharmaceutically acceptable addition salts thereof, and a pharmaceutically acceptable carrier. In certain embodiments these compositions may be formulated in unit dosage forms.

The compositions of the present invention are preferably non-pyrogenic, e.g., do not trigger elevation of a patient's body temperature by more than a clinically acceptable amount.

Plasticizers and stabilizing agents known in the art may be incorporated in the pharmaceutical compositions of the present invention. In certain embodiments, additives such as plasticizers and stabilizing agents are selected for their biocompatibility. In certain

embodiments, the additives are lung surfactants, such as 1,2-dipalmitoylphosphatidycholine (DPPC) and L-α-phosphatidylcholine (PC).

A composition of this invention may further contain one or more adjuvant substances, such as fillers, thickening agents or the like.

In certain embodiments, a subject composition includes an excipient. A particular excipient may be selected based on its melting point, solubility in a selected solvent (e.g., a solvent that dissolves the therapeutic agent), and the resulting characteristics of the microparticles.

Excipients may comprise a few percent, about 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, or higher percentage of the subject compositions.

Buffers, acids and bases may be incorporated in the subject compositions to adjust their pH. Agents to increase the diffusion distance of therapeutic may also be included.

The pharmaceutical compositions as described herein can be administered in various pharmaceutical formulations, depending on the disorder to be treated and the age, condition and body weight of the patient, as is well known in the art. For example, where the compounds are to be administered orally, they may be formulated as tablets, capsules, granules, powders or syrups; or for parenteral administration, they may be formulated as injections (intravenous, intramuscular or subcutaneous), drop infusion preparations or suppositories. For application by the ophthalmic mucous membrane route, they may be formulated as eye-drops or eye ointments. These formulations can be prepared by conventional means, and, if desired, the active ingredient may be mixed with any conventional additive, such as an excipient, a binder, a disintegrating agent, a lubricant, a solubilizing agent, a suspension aid, an emulsifying agent or a coating agent. Although the dosage will vary depending on the symptoms, age and body weight of the patient, the nature and severity of the disorder to be treated or prevented, the route of administration and the form of the drug, in general, a daily dosage of from 0.01 to 2000 mg of the compound is recommended for an adult human patient, and this may be administered in a single dose or in divided doses.

The precise time of administration and/or amount of therapeutic agent that will yield the most effective results in terms of efficacy of treatment in a given patient will depend upon the activity, pharmacokinetics, and bioavailability of a particular compound, physiological condition of the patient (including age, sex, disease type and stage, general physical condition,

responsiveness to a given dosage and type of medication), route of administration, etc. However, the above guidelines can be used as the basis for fine-tuning the treatment, e.g., determining the optimum time and/or amount of administration, which will require no more than routine experimentation consisting of monitoring the subject and adjusting the dosage and/or timing.

The phrase "pharmaceutically acceptable" is employed herein to refer to those therapeutic agents, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject chemical from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

The term "pharmaceutically acceptable salts" refers to the relatively non-toxic, inorganic and organic acid addition salts of the therapeutic agents. These salts can be prepared in situ during the final isolation and purification of the therapeutic agent, or by separately reacting a purified therapeutic agent in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride,

sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, besylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, for example, Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19)

In other cases, the compounds useful in the methods of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts" in these instances refers to the relatively non-toxic, inorganic or organic base addition salts of the compounds of the present invention. These salts can likewise be prepared in situ during the final isolation and purification of the therapeutic agent, or by separately reacting the purified therapeutic agent in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like (see, for example, Berge et al., *supra*).

When the therapeutic agent of the present invention is administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

Another aspect of the present invention is a method for preventing excessive complement activation in a patient comprising administering to said patient, a therapeutically effective amount of the compounds of the present invention.

Another aspect of the present invention is a method for treating or preventing complement-mediated tissue damage in a patient comprising administering to said patient, a therapeutically effective amount of a compound(s) of the present invention.

Another aspect of the present invention is a method for treating diseases characterized by chronic complement activation comprising administering to a patient a therapeutically effective amount of a compound(s) of the present invention. In certain embodiments, these diseases are selected from neurodegenerative diseases and pulmonary diseases. The neurodegenerative

diseases may be ones which affect the central nervous system (CNS) or the peripheral nervous system.

For example, the present compounds can be used in a method for treating complement mediated nerve myeline loss (demyelination). Myelin provides the axonal "insulation" essential for efficient neural signal conduction in both the CNS and PNS. The cell which produces myellin in the CNS is the oligodendrocyte whereas the myelin-producing cell in the PNS is the Schwann cell. Diseases characterized by demyelination occur both in the CNS and the PNS, but exhibit different etiologies. Accordingly, one aspect of the present invention is a method of treating complement mediated demyelination of nerves in the CNS or in the PNS comprising administration of a therapeutically effective amount of a compound(s) of the present invention.

In the CNS, the most common demyelination disease is multiple sclerosis (MS). While it is now widely accepted that MS is an autoimmune disease of the nervous system driven by infiltrating T cells specific for CNS antigens (See Prineas et al., (1987), Lab. Invest., 38, 409-421), there is evidence to suggest that C might be involved in myelin damage in MS. (See Yam et al., (1980), Clin. Immunol. Immunopathol., 17, 492-505; Mollenes et al., (1987), J. Neurol. Sci., 78, 17-28; Compston et al., (1989), Neuropathol. Appl. Neurobiol., 15, 307-316) Accordingly, one aspect of the present invention is a method of treating MS comprising administration of a therapeutically effective amount of a compound(s) of the present invention.

In the PNS, several neuropathies, including, Gulliain-Barre syndrome (GBS) and Miller-Fisher syndrome (MFS) are characterized by the presence of inflammation and extensive demyelination. The majority of GBS patients have serum IgM antibodies against Schwann cells and/or PNS mylelin which can, in vitro, efficiently activate the complement cascade. (See Koski et al., (1986), Ann. Neurol., 19, 573-577; Koski et al, (1990), Ann. Neurol., 27, S44-S47) Nyland et al., have shown that GBS serum or purified antibody causes C-dependent demyelination in peripheral nerve cultures. (See Nylaind et al., Acta Neurol. Scand., 58, 35-34) Moreover, it has been shown that C activation products (C3a, C5a, terminal C complex) are found in the CSF, plasma, and peripheral nerves of GBS patients. (See Hartung et al., (1987), 37, 1006-1009; Koski et al., (1987), J. Clin. Invest., 80. 1492-1497; Hays et al., (1988), J. Neuroimmuneol. 18, 231-244). Accordingly, one aspect of the present invention is a method of treating GBS or MFS comprising administration of a therapeutically effective amount of a compound(s) of the present invention.

IgM monoclonal gammopathy and peripheral neuropathy constitute other instances of PNS diseases which are associated with (aberrant) complement activation. (See Monaco et al., (1990, Peripheral neuropathy is a condition common in later stage (Type I, or Type II) diabetic patients. Accordingly, one aspect of the present invention is a method of treating IgM monoclonal gammopathy and peripheral neuropathy comprising administration of a therapeutically effective amount of a compound(s) of the present invention.

Another aspect of the present invention is a method of treating neuromuscular diseases wherein complement is implicated, comprising administering to a patient a therapeutically effective amount of a compound(s) of the present invention. An example of such neuromuscular disease is myasthenia gravis. (See Asghar SS. Pasch MC, *Frontiers in Bioscience. 5:E63-81, 2000 Sep 1.*)

Another aspect of the present invention is a method for treating Alzheimer's disease comprising administering to a patient a therapeutically effective amount of a compound(s) of the present invention. The pathological hallmark of Alzheimer's disease (AD) is the senile plaque, a proteinaceous extracellular deposit composed primarily of an amyloidogenic peptide termed R-protein, and which is surrounded by dystrophic neurites. Senile plaques are the focus of a robust and chronic inflammatory response mounted by microglia, the brain's endogenous macrophage. Eikenbloom et al. disclose complement activation in amyloid plaques in Alzheimer's dementia. (See Eikelenboom, P., Hack, C.E. et al., 1989. Virchows Archiv B - Cell Pathology Including Molecular Pathology 56, 259- 26; Eikelenboom, P., Stam, F.C., (1982) Acta Neuropathologica, 57, 239-242; see also Itagaki, S., Akiyama, H. et al., (1994), Brain Research, 645, 78-84; McGeer, P.L., Walker, D.G. et al., (1995), Abstracts of Papers of the American Chemical Society 210, 247, MEDI; McGeer, P.L., Akiyama, H. et al., (1989), Neuroscience Letters 107, 341-346; Pouplardbarthelaix, A., Dubas, F.et al., (1986), Neuropathology and Applied Neurobiology, 12, 609-610; Veerhuis, R., Janssen, I. et al., (1998a), Molecular Immunology 35, 312; Webster, S., Lue, L.F. et al., (1997b), Neurobiology of Aging 18, 415-421; Zhan, S.S., Veerhuis, R. et al., (1994), Neurodegeneration 3, 111- 117; see also references cited in Gasque et al., (2000), Immunopharmacology 49, 17I- 186). Implication of complement activation in Huntington's disease has been disclosed. (See Morgan, B.P., Gasque, P. et al., (1997), Immunopharmacology 38, 43-50, Morgan, B.P., Gasque, P., (1996), Immunology Today 17, 461-466, Morgan, B.P., Gasque, P., (1997), Clinical and Experimental Immunology 107, 1-7)

Another aspect of the present invention is a method for treating Huntington's disease (HD) comprising administering to a patient a therapeutically effective amount of a compound(s) of the present invention. Huntington's disease (HD) is an autosomal dominant inherited neurodegenerative disease characterized by the onset in mid-life of chorea, dementia, personality disturbance and inexorable progression to death. Singhrao et al. have reported significant presence of complement factors C1q, C4, C3, iC3b-neoepitope and C9-neoepitope in HD striatum, neurons, myelin and astrocytes. (See Singhrao et al., (1999), Exper. Neurolo., 159, 362–376)

Another aspect of the present invention is a method for treating Pick's disease (PD) comprising administering to a patient a therapeutically effective amount of a compound(s) of the present invention. PD is a neurodegenerative disorder, the histological hallmarks of which is the Pick body, a dense, amorphous body which is strongly stained for *tau* protein and ubiquitin. Neuronal loss and astrocyte proliferation occur in the areas of disease which appear to be restricted to the frontal and temporal lobes. Yasuhura et al. has shown that complement in implicated in Pick's disease. (See Yasuhura et al., (1994), Brain Res., 652, 346-349).

Another aspect of the present invention is a method for treating asthma comprising administering to a patient a therapeutically effective amount of a compound(s) of the present invention. Asthma is a disease that affects approximately 10% of the population. The overall annual prevalence of cases has increased by 42% in the past decade, and despite the availability of more potent and selective therapy, the annual incidence of asthma mortality has risen by 40% over this same time period. Asthma is an allergenic reaction toward an inhaled antigen, characterized by a strong bronchoconstriction and edema formation with subsequent cell infiltration into the lung parenchyma and alveoli, mainly lymphocytes and eosinophils. Although IgE mediated histamine release is generally regarded as the major pathophysiological pathway for asthma, other non-IgE mediated mechanisms also contribute to the disease. A major candidate in that respect is the C3a analphylatoxin. Other complement mediated pulmonary disorders include hypersensitivity pneumonites, and anaphylaxis. (See Regal, J., (1997), Immunopharmacology, 38, 17-25)

Another aspect of the present invention is a method for treating or preventing a selected from sepsis, adult respiratory distress syndrome, nephrites, graft rejection, myocardial ischemia/reperfusion injury, and intestinal ischemia/reperfusion injury, comprising administering

to a patient a therapeutically effective amount of a compound(s) of the present invention. Lipton et al., in U.S. Patent No. 6,503,947 discloses attenuation of cerebral ischemia and reperfusion injury by administrating a complement inhibitor.

Another aspect of the present invention is a method for antagonizing the C3a receptor in a patient by administering an effective amount of a compound(s) of the present invention.

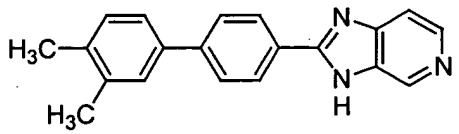
Another aspect of the present invention is a method for antagonizing the C5a receptor in a patient by administering an effective amount of a compound(s) of the present invention.

Examples

General Procedures:

All reactions were run under a nitrogen atmosphere for convenience and to maximize yields. Melting points are uncorrected. Chromatography refers to flash chromatography on silica gel. NMR refers to proton [^{1}H] NMR. NMR spectra were obtained at 400 MHz and are reported in parts per million (δ) relative to the deuterium lock signal of the solvent of the specified solvent. ^{13}C NMR spectra are referred to as such. Evaporation or concentration at reduced pressure implies the use of a rotary evaporation apparatus.

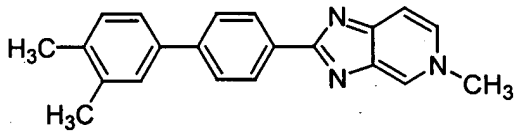
Example 1: Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine, **(1)**



3',4'-Dimethyl-biphenyl-4-carboxylic acid (0.50 g, 2.21 mmol, ~~Preparation 1~~ Example 23), 3,4-diaminopyridine (0.24 g, 2.22 mmol) and polyphosphoric acid (2g) were heated at 200°C for 2 h then poured into water and stirred vigorously. The slurry was made basic with sat. $NaHCO_3$ and the gray solid was filtered and dried (0.52g, 79%). Recrystallization from ethanol/1 N HCl gave 0.27 g of 2-(3',4'-dimethyl-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine hydrochloride as a light yellow solid which had: mp >260°C; NMR (DMSO-d_6) δ 9.38 (s, 1H), 8.53 (d, J = 7.1 Hz, 1H), 8.09 (d, J = 6.2Hz, 1H), 7.89 (d, J = 8.7 Hz, 2H), 7.55 (s, 1H), 7.48 (dd, J = 7.5, 2.3 Hz, 1H),

7.21 (d, J = 7.9 Hz, 1H), 2.27 (s, 3H), 2.24 (s, 3H); Anal. Calculated for $C_{20}H_{17}N_3 \bullet HCl \bullet 0.75\ H_2O$: C, 68.76; H, 5.41; N, 12.03. Found: C, 68.83; H, 5.43; N, 12.06.

Example 2: Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-methyl-5H-imidazo[4,5-c]pyridine, **(2)**

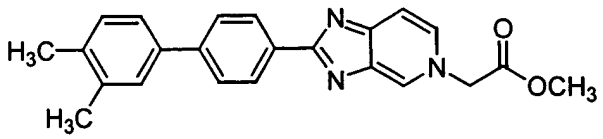


2-(3',4'-Dimethyl-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine hydrochloride (Example 1, 0.50 g, 1.49 mmol) was added to a slurry of 60% sodium hydride (0.13 g, 3.25 mmol) in DMSO (10 mL). This mixture was stirred until the solids dissolved (~15 min) and methyl iodide (0.10 mL, 1.61 mmol) was added and the reaction was stirred 60 h at rt. A white solid precipitated from solution – water (80 mL) was added and the solids were filtered and air dried (0.52g). Recrystallization from EtOAc/MeOH (6:1) with two hot filtrations gave 0.25 g (55%) of 2-(3',4'-dimethyl-biphenyl-4-yl)-5-methyl-5H-imidazo[4,5-c]pyridine as a dull yellow solid which had: mp 243-244°C; NMR (DMSO-d_6) δ 8.82 (d, J= 1.7 Hz, 1H), 8.37 (d, J= 8.3 Hz, 2H), 7.96 (dd, J = 6.6, 1.7 Hz, 1H), 7.70 (d, J = 8.7 Hz, 2H), 7.65 (d, J = 6.6 Hz, 1H), 7.50 (d, J = 1.6 Hz, 1H), 7.42 (dd, J = 7.9, 1.5 Hz, 1H), 7.19 (d, J = 7.9 Hz, 1H), 4.16 (s, 3H), 2.26 (s,3H), 2.22 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 171.29, 155.99, 146.04, 141.37, 137.82, 137.43, 136.39, 134.56, 132.75, 132.23, 130.74, 128.77, 128.28, 126.97, 124.51, 112.56, 46.28, 20.20, 19.74; Anal. Calculated for $C_{21}H_{19}N_3 \bullet 0.25\ H_2O$: C, 79.34; H, 6.18; N, 13.22. Found: C, 79.30; H, 5.97; N, 13.34.

Example 3

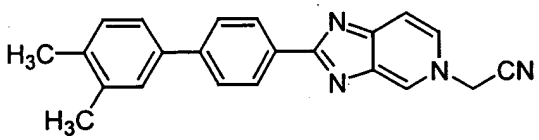
The title compounds below were all made by essentially the same procedure as exemplified in Example 2.

A. Synthesis of [2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetic acid methyl ester, **(3)**



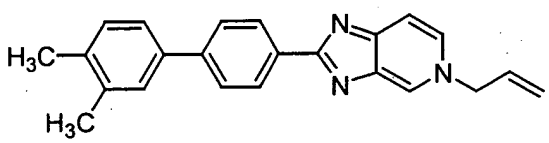
Alkylation with methyl bromoacetate - yield 76%. The maleate salt prepared in ethyl acetate had: mp 194-195°C; NMR (DMSO-d_6) δ 9.40 (s, 1H), 8.55 (dd, J = 7.1, 1.3 Hz, 1H), 8.33 (d, J = 8.7 Hz, 2H), 8.17 (d, J = 6.6 Hz, 1H), 7.90 (d, J= 8.3 Hz, 2H), 7.56 (s, 1H), 7.49 (dd, J= 7.9, 1.7 Hz, 1H), 7.23 (d, J= 7.9 Hz, 1H), 6.01 (s, 2H), 5.62 (s, 2H), 2.28 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 168.36, 167.74, 144.17, 138.28,, 137.67, 137.36, 136.88, 133.82 (broad), 130.88, 129.14, 128.49, 127.75, 124.82, 60.04, 53.65, 20.19, 19.79; Anal. Calculated for $C_{23}H_{21}N_3O_2 \bullet C_4H_4O_4 \bullet 0.25\ H_2O$: C, 66.52; H, 5.17; N, 8.62. Found: C, 66.81; H, 5.04; N, 8.40

B. Synthesis of [2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetonitrile, **(4)**



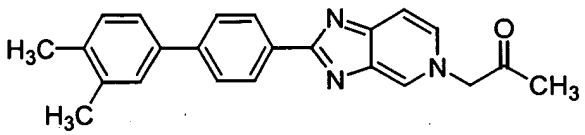
Alkylation with bromoacetonitrile – yield 87%. The maleate salt prepared in ethyl acetate/ethanol (4:1) had: mp 190-190.5°C; NMR (DMSO-d_6) δ 9.48 (s, 1H), 8.63 (dd, J = 7.1, 1.5 Hz, 1H), 8.34 (d, J= 8.7 Hz, 2H), 8.15 (d, J = 7.1 Hz, 1H), 7.89 (d, J = 8.3 Hz, 2H), 7.56 (s, 1H), 7.48 (dd, J = 7.9, 1.9 Hz, 1H), 7.23 (d, J = 7.9 Hz, 1H), 6.04 (s, 2H), 5.88 (s, 2H), 2.28 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.71, 144.00, 137.65, 137.30, 136.95, 136.49 (broad), 135.35 (broad), 130.87, 129.18, 128.48, 127.69, 124.79, 115.96, 47.16, 20.19, 19.79; Anal. Calculated for $C_{22}H_{18}N_4 \bullet C_4H_4O_4 \bullet 0.25\ H_2O$: C, 68.04; H, 4.94; N, 12.21. Found: C, 68.25; H, 4.82; N, 12.12.

C. Synthesis of 5-Allyl-2-(3',4'-dimethyl-biphenyl-4-yl)-5H-imidazo[4,5-c]pyridine, **(5)**



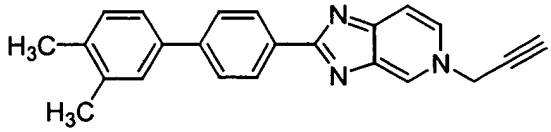
Alkylation with allyl bromide. The maleate salt had : mp 140-141°C; NMR (DMSO-d_6) δ 9.42 (s, 1H), 8.52 (d, J = 6.6 Hz, 1H), 8.32 (d, J = 8.3 Hz, 2H), 8.11 (d, J = 6.6 Hz, 1H), 7.90 (d, J = 8.3 Hz, 2H), 6.23-6.13 (sym. mult. , 1H), 5.99 (s, 2H), 5.38-5.23 (m, 4H), 2.28 (s, 3H), 2.24 (s, 3H).

D. Synthesis of 1-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-propan-2-one, **(6)**



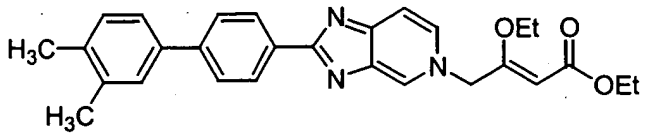
Alkylation with chloroacetone – 45% yield.. The maleate salt had: mp 172-173°C; NMR (DMSO-d_6) δ 9.24 (s, 1H), 8.40 (d, J = 6.6 Hz, 1H), 8.35 (d, J = 8.3 Hz, 2H), 8.17 (d, J = 7.1 Hz, 1H), 7.93 (d, J = 8.3 Hz, 2H), 7.59 (s, 1H), 7.51 (d, J = 7.9 Hz, 1H), 7.26 (d, J = 7.9 Hz, 1H), 6.03 (s, 2H), 5.72 (s, 2H), 2.32 (s, 3H), 2.31 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 200.89, 167.83, 143.99, 137.75, 137.31, 137.16, 136.93, 136.39 (broad), 130.87, 129.09, 128.48, 128.02. 127.71, 124.80, 111.70, 68.03, 27.65, 20.21, 19.80; Anal. Calculated for $C_{23}H_{21}N_3O \bullet C_4H_4O_4$: C, 68.78; H, 5.34; N, 8.91. Found: C, 68.71; H, 5.59; N, 8.92.

E Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-prop-2-ynyl-5H-imidazo[4,5-c]pyridine, **(7)**



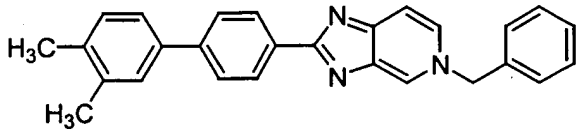
Alkylation with propargyl chloride – 42% yield. The maleate salt had: mp 148-149.5°C; NMR (DMSO-d_6) δ 9.35 (s, 1H), 8.54 (d, J = 6.2 Hz, 1H), 8.33 (d, J = 8.7 Hz, 2H), 8.11 (d, J = 6.6 Hz, 1H), 7.96-7.87 (m, 3H), 7.56 (s, 1H), 7.49 (dd, J = 7.5, 2.1 Hz, 1H), 7.23 (d, J = 7.9 Hz, 1H), 6.18 (d, J = 6.2 Hz, 2H), 6.04 (s, 2H), 2.28 (s, 3H), 2.24 (s, 3H); Anal. Calculated for $C_{23}H_{19}N_3 \bullet C_4H_4O_4 \bullet H_2O$: C, 68.78; H, 5.34; N, 8.91. Found: C, 68.79; H, 5.27; N, 8.92.

F. Synthesis of 4-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-3-ethoxy-but-2-enoic acid ethyl ester, **(8)**



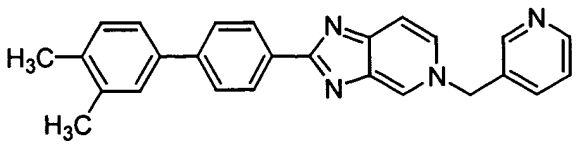
Alkylation with ethyl-4-bromo-3-ethoxy-but-2-enoate - 71% yield. A sample purified by flash chromatography (2-5% methanol/methylene chloride) followed by ethyl acetate trituration gave a white solid which had: mp 180-180.5°C; NMR ($CDCl_3$) δ 8.94 (s, 1H), 8.56 (d, J = 8.7 Hz, 1H), 8.09 (d, J = 5.8 Hz, 1H), 8.00-7.98 (m, 1H), 7.74 (d, J= 8.3 Hz, 2H), 7.43 (s, 1H), 7.39 (d, J= 7.5 Hz, 1H), 7.20 (d, J = 7.9 Hz, 1H), 5.64 (s, 2H), 5.27 (d, J = 3.8 Hz, 1H), 4.22 (q, J= 7.2 Hz, 2H), 3.85 (q, J = 6.9 Hz, 2H), 2.32 (s, 3H), 2.29 (s, 3H), 1.31 (t, J = 7.3 Hz, 6H); Anal. Calculated for $C_{28}H_{29}N_3O_3 \bullet 0.50\ H_2O$: C, 72.39; H, 6.51; N, 9.04. Found: C, 72.66; H, 6.39; N, 9.07.

G. Synthesis of 5-Benzyl-2-(3',4'-dimethyl-biphenyl-4-yl)-5H-imidazo[4,5-c]pyridine, **(9)**



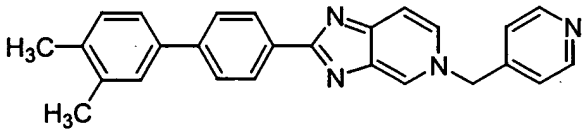
Alkylation with benzyl bromide – yield 85%. Maleate salt had: mp 177-178°C; NMR (DMSO-d_6) δ 9.65 (s, 1H), 8.69 (dd, J = 6.6, 1.2 Hz, 1H), 8.32 (d, J = 8.7 Hz, 2H), 8.11 (d, J = 6.6 Hz, 1H), 7.89 (d, J = 8.7 Hz, 2H), 7.56 (s, 1H), 7.51-7.47 (m, 3H), 7.42 –7.34 (m, 3H), 7.22 (d, J = 7.9 Hz, 1H), 6.01 (s, 2H), 5.83 (s, 2H), 2.28 (s, 3H), 2.23 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.81, 161.68, 148.21, 144.10, 141.03, 137.66, 137.34, 136.88, 136.24, 136.19, 135.57, 130.86, 129.80, 129.66, 129.04, 128.95, 128.50, 127.75, 124.79, 112.44, 63.08, 20.21, 19.80; Anal. Calculated for $C_{27}H_{23}N_3 \bullet C_4H_4O_4 \bullet 0.50\ H_2O$: C, 72.36; H, 5.48; N, 8.17. Found: C, 72.21; H, 5.57; N, 8.09

H. Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-pyridin-3-ylmethyl-5H-imidazo[4,5-c]pyridine, **(10)**



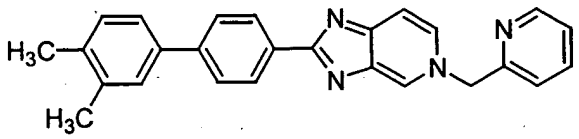
Alkylation with (3-bromomethyl) pyridine hydrobromide with 2.2 eq sodium hydride as base – yield 46%. Dimaleate salt had: mp 184-185°C; NMR (DMSO-d_6) δ 9.73 (s, 1H), 8.79-8.76 (m, 2H), 8.57 (dd, J = 4.6, 1.3 Hz, 1H), 8.31 (d, J = 8.3 Hz, 2H), 8.17 (d, J = 7.1 Hz, 1H), 7.94-7.90 (m, 3H), 7.56 (s, 1H), 7.49 (dd, J = 7.9, 1.7 Hz, 1H), 7.43 (dd, J = 7.9, 5.4 Hz, 1H), 7.23 (d, J = 7.9 Hz, 1H), 6.07 (s, 4H), 5.90 (s, 2H), 2.28 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.70, 160.71, 150.88, 150.18, 144.40, 137.69, 137.44, 137.34, 136.85, 136.78, 136.08 (broad), 134.85, 131.86, 130.88, 129.07, 128.49, 127.84, 126.99, 124.83, 124.72, 112.49, 60.80, 20.21, 19.80; Anal. Calculated for $C_{26}H_{22}N_4 \bullet 2C_4H_4O_4$: C, 65.59; H, 4.86; N, 9.00. Found: C, 65.34; H, 5.18; N 9.61

I. Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-pyridin-4-ylmethyl-5H-imidazo[4,5-c]pyridine, **(11)**



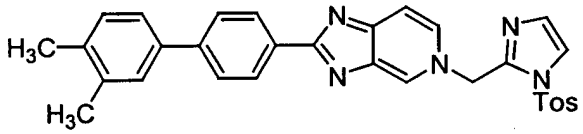
Alkylation with (4-bromomethyl) pyridine hydrobromide (*J.Org.Chem.*, **1958**, *23*, 575) with 3.4 eq sodium hydride as the base – yield 52%. Maleate salt had: mp 204.5-205°C; NMR (DMSO-d_6) δ 9.63 (s, 1H) 8.67 (d, J = 6.6 Hz, 1H), 8.58 (d, J = 5.8 Hz, 2H), 8.33 (d, J = 7.8 Hz, 2H), 8.16 (d, J = 7.1 Hz, 1H), 7.90 (d, J = 8.3 Hz, 2H), 7.56 (s, 1H), 7.48 (d, J = 7.9 Hz, 1H), 7.34 (d, J = 5.8 Hz, 2H), 7.23 (d, J = 7.9 Hz, 1H), 6.02 (s, 2H), 5.90 (s, 2H), 2.28 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.78, 161.83, 150.94, 148.39, 144.92, 144.17, 141.13, 137.67, 137.25, 136.87, 135.96, 130.87, 129.08, 128.51, 127.78, 127.65, 124.80, 122.93, 112.49, 61.69, 20.21, 19.80; Anal. Calculated for $C_{26}H_{22}N_4 \bullet C_4H_4O_4 \bullet 0.5H_2O$: C, 69.89; H, 5.28; N, 10.87. Found: C, 70.05; H, 5.21; N 10.82

J. Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-pyridin-2-ylmethyl-5H-imidazo[4,5-c]pyridine, **(12)**



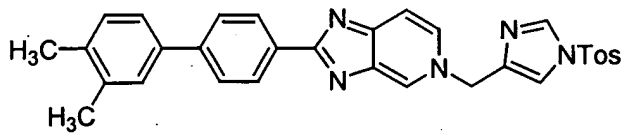
Alkylation with (2-bromomethyl) pyridine hydrobromide (prepared from 2-pyridylcarbinol following the procedure of: *J.Org.Chem.*, **1958**, *23*, 575) with 3.4 eq sodium hydride as the base – yield 41%. Maleate salt had: mp 177-178°C; NMR (DMSO-d_6) δ 9.58 (s, 1H), 8.66 (dd, J = 6.6, 1.2 Hz, 1Hθ), 8.47-8.45 (m, 1H), 8.32 (d, J = 8.7 Hz, 2H), 8.14 (d, J = 6.6 Hz, 1H), 7.92-7.85 (m, 3H), 7.57-7.54 (m, 2H), 7.49 (dd, J = 7.9, 1.7 Hz, 1H), 7.38-7.34 (sym. mult., 1H), 7.23 (d, J = 7.9 Hz, 1H), 6.00 (s, 2H), 5.99 (s, 2H), 2.28 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.77, 154.66, 150.26, 144.17, 138.27, 137.95, 137.67, 137.37, 136.87, 136.11, 130.87, 129.07, 128.51, 127.77, 127.554, 124.82, 124.46, 123.19, 112.49, 63.77, 20.21, 19.80; Anal. Calculated for $C_{26}H_{22}N_4 \bullet C_4H_4O_4 \bullet 0.5H_2O$: C, 69.89; H, 5.28; N, 10.87. Found: C, 70.11; H, 5.35; N 10.79

K. Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-[1-(toluene-4-sulfonyl)-1H-imidazol-2-ylmethyl]-5H-imidazo[4,5-c]pyridine, **(13)**



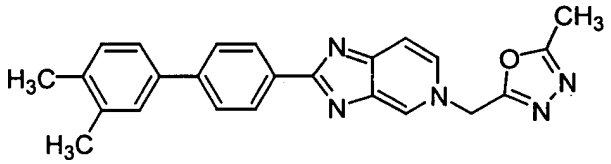
Alkylation with methanesulfonic acid 1-(toluene-4-sulfonyl)-1H-imidazol-2-ylmethyl ester (*J. Med. Chem.*, **1996**, *39*, 2907) – yield 74%. Maleate salt had: mp 167-168.5°C; NMR (DMSO-d_6) δ 9.55 (s, 1H), 8.67 (dd, J = 6.6, 1.2 Hz, 1H), 8.34 (d, J = 8.3 Hz, 2H), 8.16 (d, J = 7.1 Hz, 1H), 8.10 (d, J = 8.3 Hz, 2H), 7.93 (d, J = 8.3 Hz, 2H), 7.89 (d, J = 1.7 Hz, 1H), 7.58-7.49 (m, 4H), 7.25 (d, J = 7.9 Hz, 1H), 6.99 (d, J= 1.7 Hz, 1H), 6.29 (s, 2H), 6.04 (s, 2H), 2.42 (s, 3H), 2.30 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.77, 147.69, 144.32, 143.72, 138.67, 137.71, 137.43, 136.90, 135.82, 134.55, 131.40, 130.93, 130.08, 129.17, 128.52, 127.81, 127.39, 124.85, 122.13, 57.46, 21.85, 20.15, 19.77; Anal. Calculated for $C_{31}H_{27}N_5O_2S \bullet C_4H_4O_4 \bullet 0.5H_2O$: C, 63.86; H, 4.90; N, 10.64. Found: C, 63.45; H, 4.97; N 10.49.

L. Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-[1-(toluene-4-sulfonyl)-1H-imidazol-4-ylmethyl]-5H-imidazo[4,5-c]pyridine, **(14)**



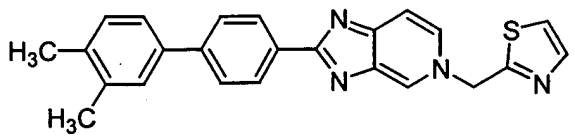
Alkylation with methanesulfonic acid 1-(toluene-4-sulfonyl)-1H-imidazol-4-ylmethyl ester (*J. Med. Chem.*, **1996**, *39*, 2907) – yield 53%. 1.5 Maleate salt had: mp 190-195°C; NMR (DMSO-d_6) δ 9.57 (s, 1H), 8.67 (dd, J = 6.6, 1.0 Hz, 1H), 8.43 (d, J = 0.8 Hz, 1H), 8.32 (d, J = 8.7 Hz, 2H), 8.15 (d, J = 6.6 Hz, 1H), 7.99-7.92 (m, 5H), 7.58 (s, 1H), 7.51-7.49 (m, 3H), 7.25 (d, J= 7.9 ~~h~~Hz, 1H), 6.11 (s, 3H), 5.78 (s, 2H), 2.39 (s, 3H), 2.30 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.66, 147.59, 144.47, 138.75, 137.72, 137.48, 136.81, 134.52, 134.13, 131.47, 130.93, 129.10, 128.57, 128.20, 127.87, 126.90, 124.89, 117.86, 56.85, 21.85, 20.24, 19.80; Anal. Calculated for $C_{31}H_{27}N_5O_2S \bullet 1.5C_4H_4O_4 \bullet 1.25H_2O$: C, 60.85; H, 4.90; N, 9.59. Found: C, 60.45; H, 4.86; N 9.49.

M. Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-(5-methyl-[1,3,4]oxadiazol-2-ylmethyl)-5H-imidazo[4,5-c]pyridine, **(15)**



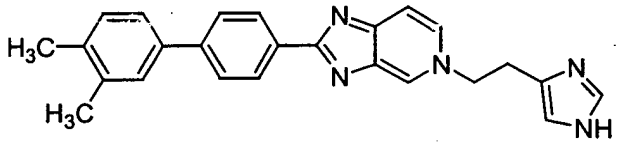
Alkylation with 2-chloromethyl-5-methyl-[1,3,4]oxadiazole (*Helv.Chim. Acta*, ***1972***, *55*, 1979) – yield 64%. Maleate salt had: mp 196-198°C; NMR (DMSO-d_6) δ 9.62 (s, 1H), 8.71 (d, J = 6.6 Hz, 1H), 8.45 (d, J = 8.3 Hz, 2H), 8.19 (d, J = 7.1 Hz, 1H), 7.88 (d, J = 8.3 Hz, 2H), 7.57 (s, 1H), 7.49 (d, J = 7.5 Hz, 1H), 7.23 (d, J = 7.9 Hz, 1H), 6.28 (s, 2H), 6.02 (s, 2H), 2.48 (s, 3H), 2.28 (s, 3H), 2.23 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.77, 165.77, 162.12, 144.25, 138.94, 137.92, 137.66, 137.34, 136.90, 135.95, 130.86, 129.35, 128.53, 127.72, 124.83, 53.57, 20.19, 19.79, 11.16.

N. Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-thiazol-2-ylmethyl-5H-imidazo[4,5-c]pyridine, **(16)**



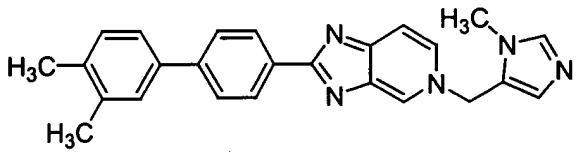
Alkylation with methanesulfonic acid thiazol-2-ylmethyl ester (~~Preparation 3~~ Example 25) – yield 64%. The maleate salt prepared in ethyl acetate had: mp 187-189°C; NMR (DMSO-d_6) δ 9.62 (s, 1H), 8.71 (d, J= 7.1 Hz, 1H), 8.32 (d, J = 8.3 Hz, 2H), 8.15 (d, J = 6.6 Hz, 1H), 7.90 (d, J = 8.3 Hz, 2H), 7.82 (AB quartet,Δ_v=9.1, J = 3.3 Hz, 2H), 7.56 (s, 1H), 7.48 (dd, J = 7.9, 1.7 Hz, 1H), 7.23 (d, J= 8.3 Hz, 1H), 6.25 (s, 2H), 6.02 (s, 2H), 2.28 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.90, 163.07, 162.05, 148.73, 144.14, 143.78, 143.70, 140.80, 137.65, 1137.49, 137.34, 136.87, 136.20, 136.15, 136.02, 130.89, 130.83, 129.11, 129.51, 128.43, 127.72, 127.66, 124.82, 124.77, 123.30, 123.26, 123.22, 112.30, 59.63, 20.20, 20.15, 19.80, 19.74; Anal. Calculated for $C_{24}H_{20}N_4S \bullet C_4H_4O_4 \bullet 1.25H_2O$: C, 62.85; H, 5.00; N, 10.47. Found: C, 62.57; H, 4.76; N, 10.29.

O. Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-[2-(1H-imidazol-4-yl)-ethyl]-5H-imidazo[4,5-c]pyridine, **(17)**



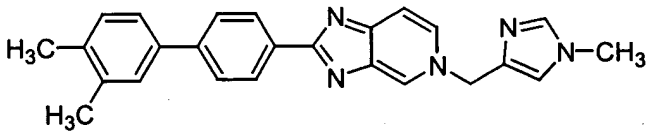
Alkylation with methanesulfonic acid 2-[1-(toluene-4-sulfonyl)-1H-imidazol-4-yl]-ethyl ester (~~Preparation 5~~ Example 27) and heating to 155° C – yield 56%. The dihydrochloride salt as a yellow solid had: mp >260°C; NMR (DMSO-d_6) δ 14.46 (s, 1H), 9.58 (s, 1H), 9.04 (d, J = 1.2 Hz, 1H), 8.62 (dd, J = 7.1, 1.3 Hz, 1H), 8.43 (d, J= 8.3 Hz, 2H), 8.13 (d, J = 6.6 Hz, 1H), 7.90 (d, J = 8.7 Hz, 2H), 7.57 (s, 1H), 7.49 (dd, J= 7.5, 1.9 Hz, 1H), 7.39 (s, 1H), 7.23 (d, J = 7.9 Hz, 1H), 5.01 (t, J = 7.1 Hz, 2H), 3.42 (t, J = 7.1 Hz, 2H), 2.8 (s, 3H), 2.24 (s, 3H); FIA- MS –394 (PH^+).

P. Synthesis of 2-(3';4'-Dimethyl-biphenyl-4-yl)-5-(3-methyl-3H-imidazol-4-ylmethyl)-5H-imidazo[4,5-c]pyridine, **(18)**



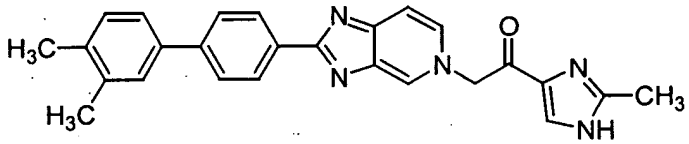
Alkylation with 5-chloromethyl-1-methyl-1H-imidazole hydrochloride (~~Preparation 8~~ Example 30) - yield 18%. The dihydrochloride salt had: mp 248-250°C; NMR (DMSO-d_6) δ 9.66 (s, 1H), 9.17 (s, 1H), 8.77 (dd, J = 6.6, 1.2 Hz, 1H), 8.42 (d, J = 8.3 Hz, 2H), 8.20 (d, J = 7.1 Hz, 1H), 7.94-7.91 (m, 3H), 7.57 (s, 1H), 7.50 (dd, J = 7.5, 1.9 Hz, 1H), 7.24 (d, J= 7.9 Hz, 1H), 6.13 (s, 2H), 3.83 (s, 3H), 2.28 (s, 3H), 2.24 (s, 3H).

Q. Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-(1-methyl-1H-imidazol-4-ylmethyl)-5H-imidazo[4,5c]pyridine, **(19)**



Alkylation with 4-chloromethyl-1-methyl-1H-imidazole hydrochloride (~~Preparation 9~~ Example 31) – yield 18%. Dihydrochloride had: mp 235-237°C (EtOH); NMR (DMSO-d_6) δ 9.70 (s, 1H), 8.99 (br s, 1H), 8.79 (d, J = 6.2 Hz, 1H), 8.41 (d, J = 8.3 Hz, 2H), 8.19 (d, J = 6.2 Hz, 1H), 7.92-7.89 (m, 3H), 7.57 (s, 1H), 7.49 (d, J = 7.9 Hz, 1H), 7.23 (d, J = 7.9 Hz, 1H), 5.96 (br s, 2H), 3.80 (s, 3H), 2.27 (s, 3H), 2.23 (s, 3H).

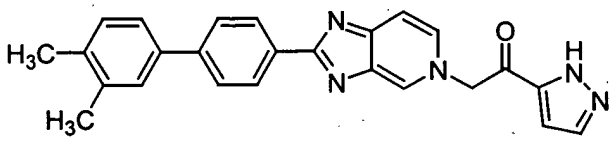
R. Synthesis of 2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2-methyl-1H-imidazol-4-yl)-ethanone, **(20)**



Alkylation with 2-bromo-1-(2-methyl-1H-imidazol-4-yl)-ethanone, hydrobromide (~~Preparation 10~~ Example 32) - yield 66%. Dihydrochloride had: mp >260°C; NMR (DMSO-d_6) δ 9.49 (s,

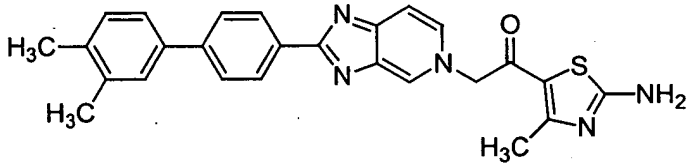
1H), 8.62 (dd, J= 6.6, 1.0 Hz, 1H), 8.51 (br s, 1H), 8.40 (d, J = 8.3 Hz, 2H), 8.24 (d, J = 6.6 Hz, 1H), 7.92 (d, J = 8.7 Hz, 2H), 7.57 (s, 1H), 7.49 (d, J= 7.9 Hz, 1H), 7.23 (d, J = 7.9 Hz, 1H), 6.27 (s, 2H), 2.53 (s, 3H), 2.28 (s, 3H), 2.23 (s, 3H); Anal. Calculated for $C_{26}H_{23}N_5O \bullet 2HCl \bullet H_2O$: C, 60.94; H, 5.31; N, 13.67. Found: C, 60.92; H, 5.43; N, 13.55.

S. Synthesis of 2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone, **(21)**



Alkylation with 2-bromo-1-(2H-pyrazol-3-yl)-ethanone (~~Preparation 11A~~ Example 33A)– yield 47%. The hydrochloride salt prepared in EtOH had: mp>260°C; ; NMR (DMSO-d_6) δ 9.48 (s, 1H), 8.62 (dd, J = 6.6, 1.2 Hz, 1H), 8.41 (d, J = 8.3 Hz, 2H), 8.21 (d, J = 6.6 Hz, 1H), 8.00 (d, J = 2.5 Hz, 1H), 7.90 (d, J= 8.3 Hz, 2H), 7.56 (s, 1H), 7.48 (dd, J= 7.9, 1.7 Hz, 1H), 7.22 (d, J = 7.9, 1.0 Hz, 1H), 6.86 (d, J= 2.5 Hz, 1H), 6.34 (s, 2H), 2.27 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 187.29, 160.31, 148.16, 144.43, 139.21, 137.67, 137.42, 136.79, 132.07, 130.86, 129.28, 127.77, 26.79, 124.83, 111.56, 106.58, 65.83, 20.17, 19.78; Anal. Calculated for $C_{25}H_{21}N_5O \bullet 2HCl$: C, 62.51; H, 4.83; N, 14.58. Found: C, 62.93; H, 5.08; N, 14.59.

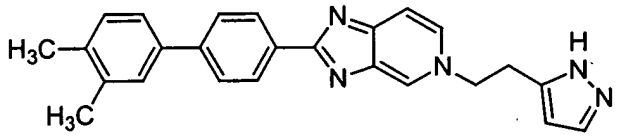
T. Synthesis of 1-(2-Amino-4-methyl-thiazol-5-yl)-2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-ethanone, **(22)**



Alkylation with 1-(2-amino-4-methyl-thiazol-5-yl)-2-bromo-ethanone (~~Preparation 11B~~ Example 33B)– yield 58%. The hydrochloride salt prepared in EtOH had : mp>260°C; NMR (DMSO-d_6) δ 9.45 (s, 1H), 8.58 (dd, J = 7.1, 1.3 Hz, 1H), 8.50 (br s, 1H), 8.46 (d, J = 8.3 Hz, 2H), 8.21 (d, J = 6.6 Hz, 1H), 7.92 (d, J = 8.3 Hz, 2H), 7.59 (s, 1H), 7.51 (d, J = 7.8 Hz, 1H), 7.24 (d, J= 7.9 Hz, 1H), 6.15 (m, 3H), 2.53 (s, 3H), 2.29 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 182.65,

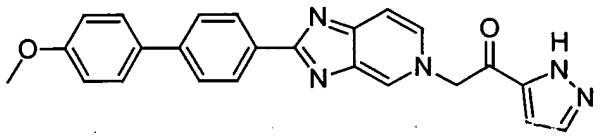
172.04, 144.43, 139.15, 137.67, 136.82, 134.40,130.89, 129.32, 128.57, 127.76, 126.82, 117.74, 66.57, 20.17, 19.78, 18.71.

U. Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-[2-(2H-pyrazol-3-yl)-ethyl]-5H-imidazo[4,5-c]pyridine, **(23)**



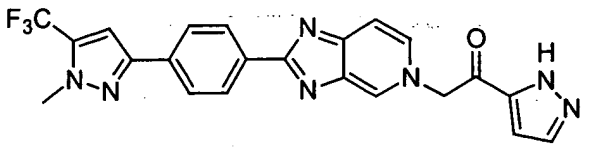
Alkylation with toluene-4-sulfonic acid 2-[2-(toluene-4-sulfonyl)-2H-pyrazol-3-yl]-ethyl ester (~~Preparation 18~~ Example 40) – yield 17%. The dihydrochloride salt prepared in ethanol had:~~:~~ mp 240-243°C; NMR (DMSO-d_6) δ 9.47 (s, 1H), 8.61 (dd, J = 6.6, 1.3 Hz, 1H), 8.39 (d, J = 8.3 Hz, 2H), 8.10 (d, J= 7.1 Hz, 1H), 7.90 (d, J = 8.7 Hz, 2H), 7.60 (d, J = 2.5 Hz, 1H), 7.57 (s, 1H), 7.49 (dd, J = 7.5, 1.9 Hz, 1H), 7.23 (d, J = 8.3 Hz, 1H), 6.12 (d, J= 2.1 Hz, 1H), 4.93 (t, J = 7.1 Hz, 2H), 3.31 (t, J = 6.8 Hz, 2H), 2.76 (s, 3H), 2.24 (s, 3H).

V. Synthesis of 2-[2-(4'-Methoxy-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone hydrochloride, **(24)**



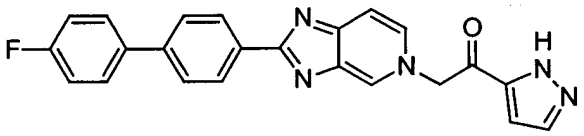
Alkylation of 2-(4'-methoxy-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine (~~Preparation 15~~ Example 37) with 2-bromo-1-(2H-pyrazol-3-yl)-ethanone (~~Preparation 11A~~ Example 33A)– yield 22%. The hydrochloride salt prepared in EtOH had: mp>260°C; NMR (DMSO-d_6) δ 9.48 (s, 1H), 8.63 (dd, J = 6.6, 1.2 Hz, 1H), 8.38 (d, J= 8.3 Hz, 2H), 8.24 (d, J = 6.6 Hz, 1H), 8.02 (d, J = 2.5 Hz, 1H), 7.91 (d, J = 8.3 Hz, 2H), 7.75 (d, J= 8.7 Hz, 2H), 7.05 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 2.5 Hz, 1H), 6.33 (s, 2H), 3.79 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 187.31, 160.34, 148.15, 144.05, 139.20, 137.34, 132.10, 131.60, 129.33, 128.79, 127.47, 126.41, 115.23, 111.61, 106.59, 65.83, 55.96; Anal. Calculated for $C_{24}H_{19}N_5O \bullet 2HCl \bullet 1.5H_2O$: C, 56.59; H, 4.75; N, 13.75. Found: C, 56.93; H, 4.51; N, 14.05.

X. Synthesis of 2-{2-[4-(1-Methyl-5-trifluoromethyl-1H-pyrazol-3-yl)-phenyl]-imidazo[4,5-c]pyridin-5-yl}-1-(2H-pyrazol-3-yl)-ethanone maleate, **(25)**



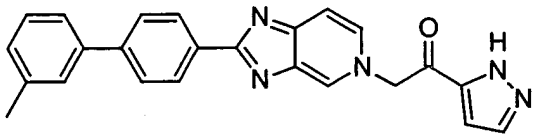
Alkylation of 2-[4-(1-methyl-5-trifluoromethyl-1H-pyrazol-3-yl)-phenyl]-3H-imidazo[4,5-c]pyridine (~~Preparation 14B~~ Example 36B) with 2-bromo-1-(2H-pyrazol-3-yl)-ethanone (~~Preparation 11A~~ Example 33A) – yield 29%. The maleate salt had: mp 215-217°C; NMR (DMSO-d_6) δ 9.41 (s, 1H), 8.56 (d, J = 6.2 Hz, 1H), 8.35 (d, J = 8.7 Hz, 2H), 8.19 (d, J = 6.6 Hz, 1H), 8.10 (d, J = 8.3 Hz, 2H), 8.03 (s, 1H), 7.57 (s, 1H), 6.88 (d, J = 2.4 Hz, 1H), 6.30 (s, 2H), 6.01 (s, 2H), 4.03 (s, 3H); Anal. Calculated for $C_{22}H_{16}F_3N_7O \bullet C_4H_4O_4$: C, 55.03; H, 3.55; N, 17.28. Found: C, 54.81; H, 3.42; N, 16.98.

Y. Synthesis of 2-[2-(4'-Fluoro-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone hydrochloride, **(26)**



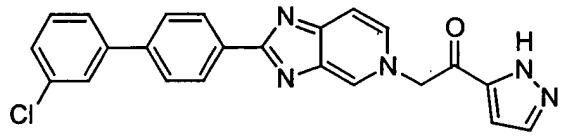
Alkylation of 2-(4'-fluoro-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine (~~Preparation 16A~~ Example 38A) with 2-bromo-1-(2H-pyrazol-3-yl)-ethanone (~~Preparation 11A~~ Example 33A) – yield 48%. The hydrochloride salt had: mp >260°C; NMR (DMSO-d_6) δ 9.51 (s, 1H), 8.64 (dd, J = 6.6, 1.2 Hz, 1H), 8.44 (d, J = 8.3 Hz, 2H), 8.24 (d, J = 6.6 Hz, 1H), 8.01 (d, J = 2.1 Hz, 1H), 7.94 (d, J = 8.3 Hz, 2H), 7.86-7.82 (m, 2H), 7.33 (t, J = 8.9 Hz, 2H), 6.88 (d, J = 1.5 Hz, 1H), 6.35 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 187.32, 164.30, 161.86, 160.15, 148.14, 147.33, 143.27, 139.24, 137.49, 135.91, 135.88, 132.03, 129.76, 129.68, 129.39, 128.10, 127.20, 116.75, 116.53, 111.64, 106.57, 65.83.

Z. Synthesis of 2-[2-(3'-Methyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone, **(27)**



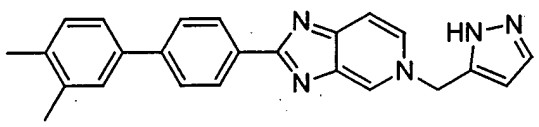
Alkylation of 2-(3'-methyl-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine(~~Preparation 16B~~ Example 38B) with 2-bromo-1-(2H-pyrazol-3-yl)-ethanone (~~Preparation 11A~~ Example 33A) – yield 10%: mp 250-260°C (decomp); NMR (DMSO-d_6) δ 8.79 (s, 1H), 8.35 (d, J = 8.3 Hz, 2H), 7.96-7.94 (m, 2H), 7.69-7.65 (m, 3H), 7.49 (s, 1H), 7.44 (d, J = 7.9 Hz, 1H), 7.28 (t, J = 7.7 Hz, 1H), 7.10 (d, J = 7.5 Hz, 1H), 6.79 (s, 1H), 6.03 (s, 2H), 2.30 (s, 3H).

AA. Synthesis of 2-[2-(3'-Chloro-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone hydrochloride, **(28)**



Alkylation of 2-(3'-chloro-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine (~~Preparation 16C~~ Example 38C) ~~)~~ with 2-bromo-1-(2H-pyrazol-3-yl)-ethanone (~~Preparation 11A~~ Example 33A) – yield 47%. The hydrochloride salt had: mp ~195-200°C; NMR (DMSO-d_6) δ 9.54 (s, 1H), 8.67 (dd, J= 7.1, 1.1 Hz, 1H), 8.49 (d, J = 8.7 Hz, 2H), 8.26 (d, J = 6.6 Hz, 1H), 8.03-8.00 (m, 3H), 7.88 (t, J = 1.9 Hz, 1H), 7.80-7.76 (m, 1H), 7.56-7.47 (m, 2H), 6.90 (d, J = 2.5 Hz, 1H), 6.37 (s, 2H).

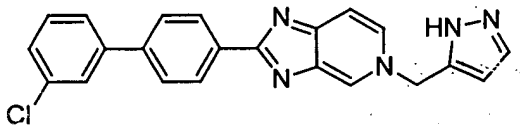
BB. Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-(2H-pyrazol-3-ylmethyl)-5H-imidazo[4,5-c]pyridine hydrochloride, **(29)**



Alkylation of 2-(3',4'-dimethyl-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine (Example 1) with 5-chloromethyl-1H-pyrazole (*JACS*, **1949**, *71*, 3994) – yield 55%. The hydrochloride salt had: mp

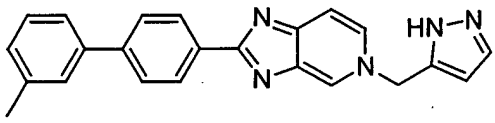
~185-200°C; NMR (DMSO-d_6) δ 9.64 (s, 1H), 8.70 (dd, J= 6.6, 1.2 Hz, 1H), 8.42 (d, J = 8.3 Hz, 2H), 8.13 (d, J = 6.6 Hz, 1H), 7.89 d, J = 8.7 Hz, 2H), 7.72 (d, J = 2.5 Hz, 1H), 7.56 (s, 1H), 7.48 (dd, J= 7.9, 1.7 Hz, 1H), 7.22 (d, J = 7.9 Hz, 1H), 6.44 (d, J= 2.5 Hz, 1H), 5.89 (s, 2H), 2.27 (s, 3H), 2.23 (s, 3H).

CC. Synthesis of 2-(3'-Chloro-biphenyl-4-yl)-5-(2H-pyrazol-3-ylmethyl)-5H-imidazo[4,5-c]pyridine hydrochloride, **(30)**



Alkylation of 2-(3'-chloro-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine (~~Preparation 16C~~ Example 38C) with 5-chloromethyl-1H-pyrazole (*JACS*, **1949**, *71*, 3994). Hydrochloride salt had: mp ~172-175°C (dec.); NMR (DMSO-d_6) δ 9.66 (s, 1H), 8.70 (d, J = 7.5 Hz, 1H), 8.43 (d, J = 8.7 Hz, 2H), 8.13 (d, J= 6.6 ~~h~~Hz, 1H), 7.93 (d, J = 8.7 Hz, 2H), 7.81 (t, J = 1.7 Hz, 1H), 7.76-7.71 (m, 2H), 7.50-7.41 (m, 2H), 6.45 (d, J = 2.1 Hz, 1H), 5.90 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 159.91, 145.59, 142.61, 141.51, 137.68, 136.09, 134.58, 131.58, 131.50, 129.32, 128.84, 128.34, 127.79, 127.29, 126.27, 112.18, 105.13, 57.19.

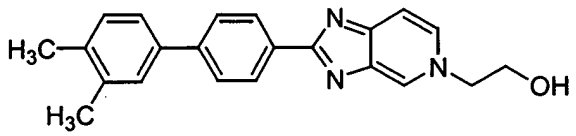
DD. Synthesis of 2-(3'-Methyl-biphenyl-4-yl)-5-(2H-pyrazol-3-ylmethyl)-5H-imidazo[4,5-c]pyridine hydrochloride, **(31)**



Alkylation of 2-(3'-methyl-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine (~~Preparation 16B~~ Example 38B) with 5-chloromethyl-1H-pyrazole (*JACS*, 1949, *71*, 3994). Hydrochloride salt had: mp ~164-167°C; NMR (DMSO-d_6) δ 9.65 (s, 1H), 8.70 (dd, J = 6.6, 1.2 Hz, 1H), 8.42 (d, J = 8.7 Hz, 2H), 8.12 (d, J= 6.6 Hz, 1H), 7.88 (d, J= 8.7 Hz, 2H), 7.72 (d, J= 2.5 Hz, 1H), 7.56 (s, 1H), 7.52 (d, J= 8.3 Hz, 1H), 7.33 (t, J = 7.7 Hz, 1H), 7.18 (d, J= 7.5 Hz, 1H), 6.45 (d, J = 2.5 Hz, 1H), 5,89 (s, 2H), 2.34 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 160.13, 147.09, 145.58, 144.41, 139.32,

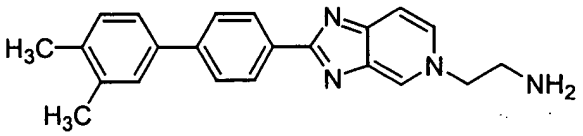
138.97, 137.64, 135.87, 131.51, 129.65, 129.27, 128.16, 128.07, 127.10,124.65, 112.13, 105.13, 57.15, 21.76.

Example 4: Synthesis of 2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-ethanol, **(32)**



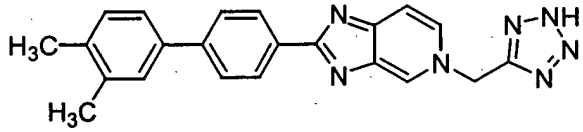
A slurry of [2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetic acid methyl ester (2.92 g, 7.86 mmol, Example 3A) in THF (150 mL) was added dropwise over 10 min. to a stirring slurry of lithium aluminum hydride (0.30 g, 7.91 mmol) in THF (50 mL)at rt. Two additional 100 mg portions of LAH were added at 1h and 2h. The reaction was carefully quenched with sodium sulfate decahydrate, then dried with anh. sodium sulfate. The mixture was filtered through Celite and the filter pad rinsed with 4:1 methylene chloride/ methanol (150 mL). The filtrate was concentrated onto silica gel and purified by flash chromatography, flushing first with methylene chloride and 5% methanol/ methylene chloride than eluting with 10% methanol/ methylene chloride to give 1.35 g (50%) of 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-ethanol as a yellow-orange solid which had: mp >260°C; NMR (DMSO-d_6) δ 8.81 (d, J = 1.3 Hz, 1H), 8.37 (d, J = 8.7 Hz, 2H), 7.98 (dd, J = 6.6, 1.5 Hz, 1H), 7.70 (d, J = 8.7 Hz, 2H), 7.65 (d, J = 6.6 Hz, 1H), 7.50 (s, 1H), 7.42 (dd, J = 7.9, 2.1 Hz, 1H), 7.19 (d, J = 7.9 Hz, 1H), 4.43 (t, J = 5.0 Hz, 2H), 3.80-3.78 (m, 2H), 3.29 (s, 3H), 2.26 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 171.06, 156.23, 145.76, 141.43, 137.85, 137.44, 136.40, 134.48, 132.55, 132.03, 130.75, 128.78, 128.28, 127.00, 124.52, 112.35, 61.52, 20.22, 20.19, 19.74; Anal. Calculated for $C_{22}H_{21}N_3O \bullet 0.50\ H_2O$: C, 74.97; H, 6.29; N, 11.92. Found: C, 74.56; H, 6.21; N, 12.11.

Example 5: Synthesis of 2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-ethylamine, **(33)**



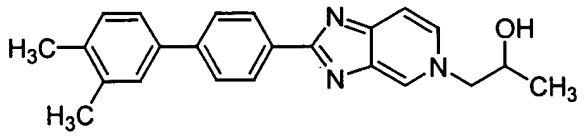
The title compound was made by essentially the same procedure as exemplified in Example 4 from [2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetonitrile (Example 3B). Dimaleate salt: mp 165-166°C; NMR (MeOH-d_4) δ 9.26 (s, 1H), 8.53 (d, J = 6.6 Hz, 1H), 8.30 (d, J= 8.7 Hz, 2H), 8.09 (d, J = 7.1 Hz, 1H), 7.85 (d, J = 8.7 Hz, 2H), 7.49 (s, 1H), 7.43 (dd, J = 7.9, 2.1 Hz, 1H), 7.23 (d, J = 7.9 Hz, 1H), 6.21 (s, 4H), 4.94 (t, J = 6.0 Hz, 2H), 3.68 (t, J = 6.0 Hz, 2H), 2.34 (s, 3H), 2.30 (s, 3H); Anal. Calculated for $C_{22}H_{22}N_4 \bullet 2C_4H_4O_4 \bullet 0.75\ H_2O$: C, 61.27; H, 5.40; N, 9.53. Found: C, 61.13; H, 5.45; N, 9.56.

Example 6: Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-(1H-tetrazol-5-ylmethyl)-5H-imidazo[4,5-c]pyridine, **(34)**



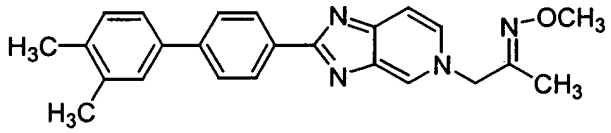
[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetonitrile (0.100 g, 0.295 mmol, Example 3B) and azidotrimethyl tin (0.125 g, 0.607 mmol) were refluxed in THF (10 mL) for 18 h. The reaction was cooled and a light tan solid was filtered off. This solid was slurried in methylene chloride (15 mL) and saturated with HCl gas. After stirring for 90 min, the light tan-yellow solid was filtered off to give 77 mg (63%) of 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(1H-tetrazol-5-ylmethyl)-5H-imidazo[4,5-c]pyridine dihydrochloride salt which had: mp 220-230°C (foams and melts); NMR (DMSO–d_6) δ 9.69 (s, 1H), 8.77 (dd, J = 6.6, 1.3 Hz, 1H), 8.39 (d, J = 8.7 Hz, 2H), 8.24 (d, J = 6.6 Hz, 1H), 7.92 (d, J = 8.7 Hz, 2H), 7.58 (s, 1H), 7.50 (dd, J = 7.9, 1.9 Hz, 1H), 7.24 (d, J = 8.3 Hz, 1H), 6.34 (s, 2H), 2.28 (s, 3H), 2.24 (s, 3H); Anal. Calculated for $C_{22}H_{19}N_7 \bullet 2HCl$: C, 58.16; H, 4.66; N, 21.58. Found: C, 57.92; H, 4.89; N, 21.38.

Example 7: Synthesis of 1-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-propan-2-ol, **(35)**



Sodium borohydride (0.043 g, 1.14 mmol) was added to a solution of 1-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-propan-2-one (0.200 g, 0.563 mmol, Example 3D) in ethanol (10 mL) and the mixture stirred at rt for 16 h. A white solid had precipitated from solution – water (20 mL) was added and the mixture was heated nearly to reflux and stirred for 15 min. The solids were filtered off, rinsed with water and dried to give 0.171 g (85%) of 1-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-propan-2-ol as a light yellow solid. The maleate salt had: mp 212-213°C; NMR (DMSO–d_6) δ 9.35 (s, 1H), 8.52 (d, J = 7.9 Hz, 1H), 8.32 (d, J = 8.7 Hz, 2H), 8.11 (d, J = 6.6 Hz, 1H), 7.90 (d, J = 8.3 Hz, 2H), 7.56 (s, 1H), 7.48 (dd, J = 7.9, 1.7 Hz, 1H), 7.23 (d, J = 7.9 Hz, 1H), 5.99 (s, 2H), 5.18 (d, J = 4.1 Hz, 1H), 4.66 (dd, J = 12.9, 2.9 Hz, 1H), 4.34 (dd, J= 13.3, 8.5 Hz, 1H), 4.05 (br s, 1H), 2.28 (s, 3H), 2.24 (s, 3H), 1.15 (d, J = 6.2 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 167.80, 144.17, 137.77, 137.68, 137.38, 136.86, 136.29 (broad), 130.87, 129.02, 128.48, 127.78, 127.45, 124.82, 111.46, 66.76, 66.60, 21.12, 20.21, 19.80; Anal. Calculated for $C_{23}H_{23}N_3O \bullet C_4H_4O_4$: C, 68.48; H, 5.75; N, 8.87. Found: C, 68.46; H, 5.92; N, 8.87.

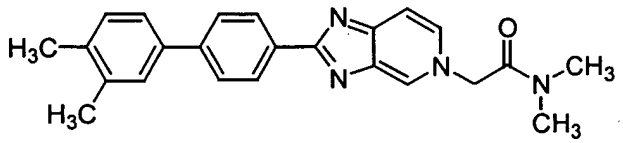
Example 8: Synthesis of 1-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-propan-2-one O-methyl-oxime, **(36)**



1-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-propan-2-one (0.100 g, 0.281 mmol, Example 3D) and methoxylamine hydrochloride (0.50 g, 0.599 mmol) in pyridine (4 mL) ~~was~~ were heated to 75°C and stirred for 3 h. The reaction was concentrated and the residue filtered and washed with water. The resulting solid was dissolved in hot ethyl acetate, washed with water and brine, dried over magnesium sulfate and concentrated. This solid was triturated with ether to give a 0.067 g (62%) of a white solid which proton NMR showed to be a 4:1 mixture of trans and cis isomers of 1-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-

yl]-propan-2-one O-methyl-oxime. The maleate salt prepared by precipitation from an ethyl acetate solution was a 5:1 mixture of trans and cis isomers which had: mp 152-154°C; NMR (DMSO–d_6) δ *trans* oxime: 9.40 (s, 1H), 8.49 (d, J = 6.6 Hz, 1H), 8.37 (d, J= 8.7 Hz, 2H), 8.12 (d, J = 6.6 Hz, 1H), 7.90 (d, J = 8.3 Hz, 2H), 7.56 (s, 1H), 7.49 (dd, J = 7.9, 1.7 Hz, 1H), 7.23 (d, J = 8.3 Hz, 1H), 6.00 (s, 2H), 5.39 (s, 2H), 3.65 (s, 3H), 2.28 (s, 3H), 2.24 (s, 3H), 1.85 (s, 3H). *cis* oxime: δ 9.43 (s, 1H), 8.55 (d, J = 6.6 Hz, 1H), 8.33 (d, J= 8.7 Hz, 2H), 8.12 (d, J = 6.6 Hz, 1H), 7.90 (d, J = 8.3 Hz, 2H), 7.56 (s, 1H), 7.49 (dd, J= 7.9, 1.7 Hz, 1H), 7.23 (d, J= 8.3 Hz, 1H), 6.00 (s, 2H), 5.54 (s, 2H), 3.84 (s, 3H), 2.28 (s, 3H), 2.24 (s, 3H), 1.62 (s, 3H).

Example 9: Synthesis of 2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-N,N-dimethyl-acetamide, **(37)**

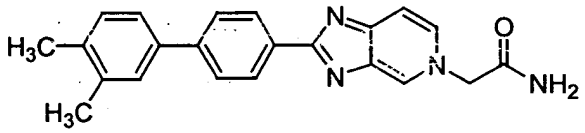


Trimethylaluminum (2M in toluene, 0.34 mL, 0.68 mmol) was added to a slurry of dimethyl amine hydrochloride (0.056 g, 0.69 mmol) in benzene (2 mL). This mixture was stirred until the solids dissolved, then a slurry of [2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetic acid methyl ester (0.100 g, 0.269 mmol, Example 3A) in benzene (8 mL) was added and the resulting mixture was refluxed for 3h. The reaction was cooled, made acidic with ~5 mL 1N HCl then brought back to ~ pH 8 with sat. sodium bicarbonate solution. The suspended solid was filtered then loaded on silica gel and purified by flash chromatography using 20% methanol/ methylene chloride as eluent to give 0.092 g (89%) of 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-N,N-dimethyl-acetamide as a white solid. The maleate salt prepared in hot ethanol had: mp 221-222°C; NMR (DMSO–d_6) δ 9.31 (s, 1H), 8.46 (d, J = 7.9 Hz, 1H), 8.35 (d, J = 8.3 Hz, 2H), 8.16 (d, J = 7.1 Hz, 1H), 7.93 (d, J =8.3 Hz, 2H), 7.59 (s, 1H), 7.52 (dd, J = 7.5, 1.9 Hz, 1H), 7.26 (d, J = 7.9 Hz, 1H), 6.02 (s, 2H), 5.70 (s, 2H), 3.09 (s, 3H), 2.92 (s, 3H), 2.31 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.94, 144.18, 138.64, 137.68, 137.37, 136.87, 136.17, 130.89, 129.10, 128.51, 127.76, 127.51, 124.82, 61.06, 36.42, 36.09, 20.21, 19.79: Anal. Calculated for $C_{24}H_{24}N_4O \bullet C_4H_4O_4$: C, 67.19; H, 5.64; N, 11.19. Found: C, 67.05; H, 5.72; N, 11.16.

Example 10

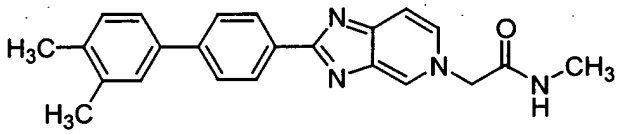
The title compounds below were made by essentially the same procedure as exemplified in Example 9.

A. Synthesis of 2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetamide, **(38)**



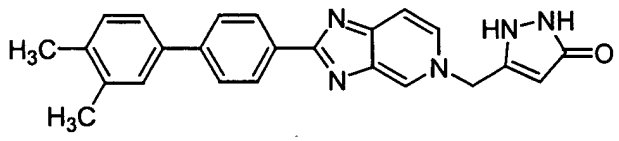
Half maleate salt: mp 223-225°C; NMR (DMSO–d_6) δ 9.37 (s, 1H), 8.51 (d, J = 7.1 Hz, 1H), 8.33 (d, J = 8.7 Hz, 2H), 8.12 (d, J = 6.6 Hz, 1H), 7.95 (s, 1H), 7.91 (d, J= 8.7 Hz, 1H), 7.61 (s, 1H), 7.57 (s, 1H), 7.49 (d, J = 7.9 Hz, 1H), 7.23 (d, J= 7.9 Hz, 1H), 5.99 (s, 1H), 5.35 (s, 2H), 2.28 (s, 3H), 2.24 (s, 3H).

B. Synthesis of 2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-N-methyl-acetamide, **(39)**



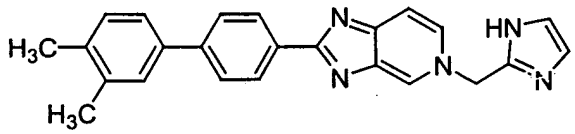
Maleate salt: mp 120-150°C (amorphous); NMR (DMSO–d_6) δ 9.41 (s, 1H), 8.54 (dd, J = 7.1, 1.0 Hz, 1H), 8.48 (q, J = 4.8 Hz, 1H), 8.36 (d, J = 8.7 Hz, 2H), 8.14 (d, J = 6.6 Hz, 1H), 7.91 (d, J= 8.3 Hz, 2H), 7.57 (s, 1H), 7.49 (dd, J= 7.9, 1.7 Hz, 1H), 7.23 (d, J= 7.9 Hz, 1H), 5.39 (s, 2H), 2.66 (d, J= 4.6 Hz, 3H), 2.28 (s, 3H), 2.24 (s, 3H).

Example 11: Synthesis of 5-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-1,2-dihydro-pyrazol-3-one, **(40)**



4-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-3-ethoxy-but-2-enoic acid ethyl ester (0.100 g, 0.220 mmol, Example 3F) and hydrazine hydrate (1 mL) in ethanol (2 mL) were refluxed for 2.5 h. The reaction was concentrated and the residue washed with water and dried. Treatment of this solid with maleic acid in ethanol ~~to give~~ gave 62 mg (55%) of 5-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-1,2-dihydro-pyrazol-3-one maleate salt as a tan solid which had: mp – foams at ~240°C; NMR (DMSO–d_6) δ 9.53 (s, 1H), 8.61 (d, J = 6.2 Hz, 1H), 8.34 (d, J= 8.3 Hz, 2H), 8.13 (d, J = 7.1 Hz, 1H), 7.93 (d, J = 8.3 Hz, 2H), 7.59 (s, 1H), 7.51 (dd, J = 7.9, 1.7 Hz, 1H), 7.26 (d, J= 7.9 Hz, 1H), 6.04 (s, 2H), 5.70 (s, 2H), 5.59 (s, 1H), 2.31 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.82, 144.22, 137.67, 137.39, 137.02, 136.83, 136.03, 130.88, 129.05, 128.49, 127.78, 127.36, 124.81, 112.12, 20.19, 19.80; Anal. Calculated for $C_{24}H_{21}N_5O \bullet C_4H_4O_4 \bullet 0.5\ H_2O$: C, 64.61; H, 5.03; N, 13.45. Found: C, 64.60; H, 4.99; N, 13.54.

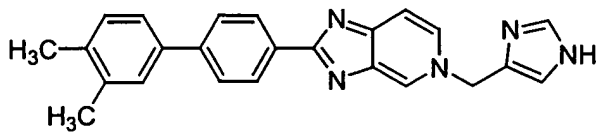
Example 12: Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-(1H-imidazol-2-ylmethyl)-5H-imidazo[4,5-c]pyridine, **(41)**



A saturated solution of HCl in ether (10 mL) was added to solution of 2-(3',4'-dimethyl-biphenyl-4-yl)-5-[1-(toluene-4-sulfonyl)-1H-imidazol-2-ylmethyl]-5H-imidazo[4,5-c]pyridine (0.100 g, 0.187 mmol, Example 3K) in methylene chloride (10 mL). After stirring at rt for 16 h, the mixture was resaturated with HCl gas and stirred for an additional 2h. The yellow precipitate was filtered off and pulped with 2mL ethanol, cooled and collected to give 40 mg (47%) of the tri-HCl salt of 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(1H-imidazol-2-ylmethyl)-5H-imidazo[4,5-c]pyridine as a yellow solid which had: mp 235-236°C; NMR (DMSO-d_6) δ 9.87 (s, 1H), 8.95 (dd, J = 6.6, 1.2 Hz, 1H), 8.44 (d, J = 8.3 Hz, 2H), 8.27 (d, J = 7.1Hz, 1H), 7.94 (d, J = 8.7 Hz, 2H), 7.72 (s, 2H), 7.60 (s, 1H), 7.52 (dd, J = 7.9, 1.7 Hz, 1H), 7.26 (d, J = 7.9 Hz, 1H), 6.31 (s,

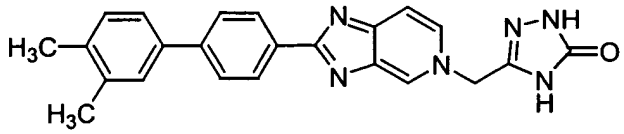
2H), 2.30 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 114.60, 140.46, 138.28, 137.68, 137.47, 136.77, 130.90, 130.85, 129.35, 128.59, 128.51, 127.81, 126.63, 124.89, 124.83, 120.90, 120.81, 112.49, 52.73, 20.16, 19.76; Anal. Calculated for $C_{24}H_{21}N_5$•3HCl•0.75 H_2O: C, 57.38; H, 5.12; N, 13.94. Found: C, 57.43; H, 5.31; N, 13.82.

Example 13: Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-(1H-imidazol-4-ylmethyl)-5H-imidazo[4,5-c]pyridine, **(42)**



Prepared following the procedure exemplified in Example 12 from 2-(3',4'-dimethyl-biphenyl-4-yl)-5-[1-(toluene-4-sulfonyl)-1H-imidazol-4-ylmethyl]-5H-imidazo[4,5-c]pyridine, Example 3L) – yield 67% of a yellow solid which had: mp >260°C; NMR (DMSO-d_6) δ 9.75 (s, 1H), 8.90 (s, 1H), 8.83 (d, J = 7.9 Hz, 1H), 8.43 (d, J = 8.3 Hz, 2H), 8.21 (d, J = 6.6 Hz, 1H), 7.93 (d, J = 8.3 Hz, 2H), 7.85 (s, 1H), 7.59 (s, 1H), 7.52 (dd, J = 7.9, 1.7 Hz, 1H), 7.26 (d, J = 7.9 Hz, 1H), 6.02 (s, 2H), 2.30 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 144.49, 137.67, 137.43, 136.78, 136.15, 130.85, 129.29, 128.58, 128.50, 128.18, 127.79, 126.71, 124.88, 120.47, 120.41, 53.24, 20.22, 20.16, 19.82, 19.76; Anal. Calculated for $C_{24}H_{21}N_5$•2HCl•0.5 H_2O: C, 62.48; H, 5.24; N, 15.18. Found: C, 62.44; H, 5.22; N, 15.12.

Example 14: Synthesis of 5-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-2,4-dihydro-[1,2,4]triazol-3-one, **(43)**

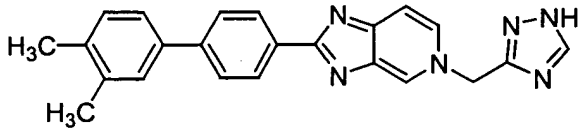


2-(3',4'-Dimethyl-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine (0.25 g, 0.744 mmol, Example 1) was added to a slurry of 60% sodium hydride (0.0663 g, 1.65 mmol) in DMSO (10 mL). This mixture was stirred until the solids dissolved (~20 min) and N'-(2-chloro-1-imino-ethyl)-hydrazinecarboxylic acid methyl ester (*J.Med Chem.* **1996**, *39*, 2907) (0.134 g, 0.809 mmol) was added and the reaction was stirred 16 h at rt.. The reaction was diluted with water (100 mL)

and the light yellow-tan solid was filtered off. This was pulped with ethyl acetate (25 mL) to give 0.183 g (57%) of N'-{2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-imino-ethyl}-hydrazinecarboxylic acid methyl ester as a light tan solid which had: NMR (DMSO-d_6) δ 9.17 (br s, 1H), 8.85 (d, J = 1.2 Hz, 1H), 8.40 (d, J = 8.7 Hz, 2H), 7.99 (dd, J = 6.6, 1.2 ~~h~~Hz, 1H), 7.74 (d, J = 8.7 Hz, 2H), 7.71 (d, J = 6.6 Hz, 1H), 7.54 (s, 1H), 7.46 (dd, J= 7.5, 1.7 Hz, 1H), 7.23 (d, J= 7.9 Hz, 1H), 6.37 (s, 2H), 5.02 (s, 2H), 3.56 (s, 3H), 2.30 (s, 3H), 2.26 (s, 3H).

This material was mixed with potassium carbonate (0.12 g, 0.87 mmol) in DMF (3.5 mL) and heated between 140° and 160°C for 1h. After concentration, the residue was triturated with water and ethyl acetate to give 0.052 g (31%) of 5-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-2,4-dihydro-[1,2,4]triazol-3-one as a tan solid. Treatment with maleic acid in ethanol/ethyl acetate followed by concentration and ethyl acetate trituration gave the ~90-95% pure maleate salt as a white solid which had: mp > 260°C; NMR (DMSO-d_6) δ 11.80 (br s, 1H), 11.62 (s, 1H), 9.50 (s, 1H), 8.59 (d, J = 6.6 Hz, 1H), 8.37 (d, J = 8.3 Hz, 2H), 8.17 (d, J = 6.6 Hz, 1H), 7.93 (d, J = 8.3 Hz, 2H), 7.59 (s, 1H), 7.52 (d, J = 7.5 Hz, 1H), 7.26 (d, J = 7.9 Hz, 1H), 6.04 (s, 2H), 5.78 (s, 2H), 2.31 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.78, 156.58, 144.25, 142.98, 137.67, 137.38, 136.87, 135.88, 130.92, 129.16, 128.55, 128.47, 127.77, 127.49, 124.86, 112.51, 55.22, 20.22, 19.82.

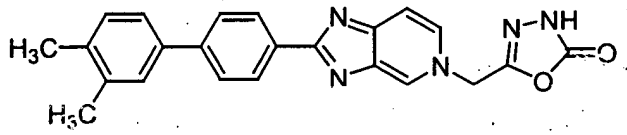
Example 15: Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-(1H-[1,2,4]triazol-3-ylmethyl)-5H-imidazo[4,5c]pyridine, **(44)**



The title compound below was made by essentially the same procedure as exemplified in Example 14 using formic acid N'-(2-chloro-1-imino-ethyl)-hydrazide (~~Preparation 2~~ Example 24) as alkylating agent. The maleate salt had: mp 227-230°C; NMR (DMSO-d_6) δ 14.24 (br s, 1H), 9.58 (s, 1H), 8.68 (d, J = 5.8 Hz, 1H), 8.59 (br s, 1H), 8.32 (d, J = 8.3 Hz, 2H), 8.15 (d, J = 7.1 Hz, 1H), 7.91 (d, J = 8.7 Hz, 2H), 7.57 (s, 1H), 7.49 (dd, J = 7.5, 2.1 Hz, 1H), 7.24 (d, J= 7.9

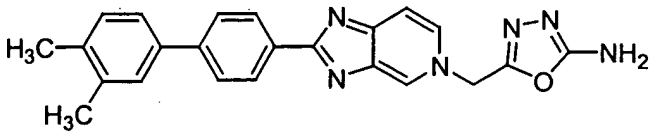
Hz, 1H), 6.02 (s, 2H), 5.99 (s, 2H), 2.28 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.78, 144.27, 137.67, 137.40, 136.84, 136.38, 135.82, 130.85, 129.11, 128.54, 128.46, 127.77, 127.34, 124.80, 112.09, 56.95, 20.21, 19.82;~~;~~ Anal. Calculated for $C_{23}H_{20}N_6 \bullet C_4H_4O_4 \bullet H_2O$: C, 63.03; H, 5.09; N, 16.33. Found: C, 62.71; H, 4.94; N, 16.44.

Example 16: Synthesis of 5-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-3H-[1,3,4]oxadiazol-2-one, **(45)**



2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetic acid hydrazide (0.20 g, 0.538 mmol, ~~Preparation 4~~ Example 26), triphosgene (0.44 g, 1.48 mmol) and triethylamine (0.31 mL, 2.22 mmol) were refluxed in THF (25 mL) for 3h. After cooling, the yellow solid was collected and triturated with ethanol to give 0.161g (64%) of 5-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-3H-[1,3,4]oxadiazol-2-one, the dihydrochloride salt of which had: mp >260°C; NMR (DMSO-d_6) δ 12.66 (s, 1H), 9.60 (s, 1H), 8.71 (dd, J = 6.6, 1.5 Hz, 1H), 8.41 (d, J = 8.7 Hz, 2H), 8.23 (d, J = 6.6 Hz, 1H), 7.92 (d, J = 8.7 Hz, 2H), 7.58 (s, 1H), 7.50 (dd, J = 7.9, 1.9 Hz, 1H), 7.24 (d, J = 8.3 Hz, 1H), 5.96 (s, 2H), 2.28 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 195.84, 160.73, 155.08, 152.04, 144.59, 138.56, 137.68, 137.46, 136.77, 130.90, 129.37, 128.58, 128.51, 127.80, 126.64, 124.88, 112.23, 54.36, 20.21, 20.16, 19.81, 19.76; Anal. Calculated for $C_{23}H_{19}N_5O \bullet 2HCl \bullet 0.25H_2O$: C, 58.17; H, 4.56; N, 14.75. Found: C, 58.23; H, 4.76; N, 14.87.

Example 17: Synthesis of 5-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-[1,3,4]oxadiazol-2-ylamine, **(46)**

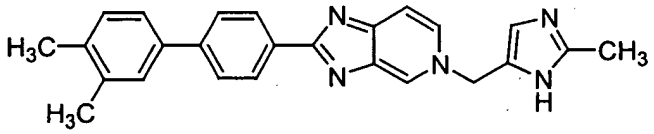


2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetic acid hydrazide (0.100 g, 0.269 mmol, ~~Preparation 4~~ Example 26) and sodium carbonate (0.029 g, 0.274 mmol) in water (1 mL)/ dioxane (5 mL) were heated to ~40-50°C for 1h. The resulting yellow solution was cooled to rt, bromocyanogen (5M in CH_3CN, 0.054 mL, 0.27 mmol) was added and the mixture was stirred for 16h. The reaction was concentrated and triturated with water, ethyl acetate and methylene chloride to give 0.085 g (80%) of 5-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-[1,3,4]oxadiazol-2-ylamine as a brown solid. The maleate salt prepared in ethanol, concentrated and triturated with ethyl acetate had: mp 235-245°C; NMR (DMSO-d_6) δ 9.50 (s, 1H), 8.61 (d, J = 6.6 Hz, 1H), 8.34 (d, J = 8.3 Hz, 2H), 8.15 (d, J = 6.6 Hz, 1H), 7.90 (d, J = 8.3 Hz, 2H), 7.57 (s, 1H), 7.49 (d, J = 7.9 Hz, 1H), 7.24-7.23 (m, 3H), 6.02 (s, 2H), 6.01 (s, 2H), 2.28 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.78, 165.30, 154.62, 144.14, 138.94, 137.67, 137.34, 136.91, 135.78, 130.85, 129.15, 128.53, 127.74, 124.83, 112.49, 53.72, 20.22, 19.82; Anal. Calculated for $C_{23}H_{20}N_6O \bullet C_4H_4O_4 \bullet 2.5H_2O$: C, 58.16; H, 5.24; N, 15.07. Found: C, 58.35; H, 4.84; N, 15.16.

Example 18

The title compounds below were made by essentially the alkylation procedures shown in Example 2 followed by the tosylate hydrolysis procedure as described in Example 12.

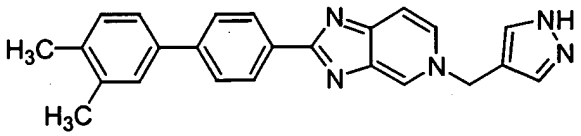
A. Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-(2-methyl-1H-imidazol-4-ylmethyl)-5H-imidazo[4,5c]pyridine, **(47)**



Alkylation with 2-methyl-1-(toluene-4-sulfonyl)-1H-imidazol-4-ylmethyl ester (~~Preparation 6~~ Example 28) – yield 71%. Dihydrochloride as a yellow solid had: mp >260°C; NMR (DMSO-d_6) δ 9.73 (s, 1H), 8.81 (dd, J= 6.6, 1.0 Hz, 1H), 8.42 (d, J = 8.3 Hz, 2H), 8.20 (d, J = 6.6 Hz, 1H), 7.91 (d, J= 8.3 Hz, 2H), 7.76 (s, 1H), 7.57 (s, 1H), 7.49 (dd, J= 7.9, 1.9 Hz, 1H), 7.23 (d, J = 7.9

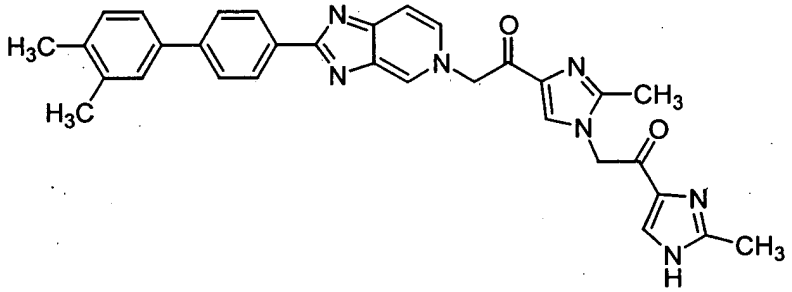
Hz, 1H), 5.99 (s, 2H), 2.50 (s, 3H), 2.28 (s, 3H), 2.24 (s, 3H); Anal. Calculated for $C_{25}H_{23}N_5 \bullet 2HCl \bullet 0.5H_2O$: C, 63.16; H, 5.51; N, 14.73. Found: C, 63.37; H, 5.33; N, 14.67

B. Synthesis of 2-(3',4'-Dimethyl-biphenyl-4-yl)-5-(1H-pyrazol-4-ylmethyl)-5H-imidazo[4,5-c]pyridine, **(48)**



Alkylation with methanesulfonic acid 1-(toluene-4-sulfonyl)-1H-pyrazol-4-ylmethyl ester (~~Preparation 7~~ Example 29). Dihydrochloride, a light yellow solid, had: mp 226-232°C; NMR (DMSO-d_6) δ 9.67 (s, 1H), 8.74 (dd, J= 6.6, 1.2 Hz, 1H), 8.39 (d, J = 8.3 Hz, 2H), 8.12 (d, J = 6.6 Hz, 1H), 7.91-7.88 (m, 4H), 7.56 (s, 1H), 7.49 (dd, J= 7.9, 2.1 Hz, 1H), 7.23 (d, J= 8.3 Hz, 1H), 5.76 (s, 2H), 2.28 (s, 3H), 2.24 (s, 3H).

Example 19. Synthesis of 2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-{2-methyl-1-[2-(2-methyl-1H-imidazol-4-yl)-2-oxo-ethyl]-1H-imidazol-4-yl}-ethanone, **(49)**



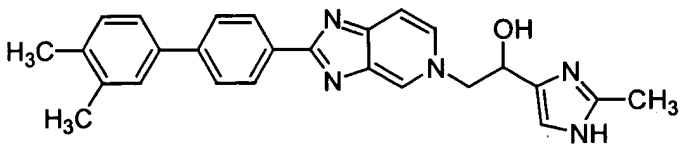
2-(3',4'-Dimethyl-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine hydrochloride (4.14 g, 12.33 mmol, ~~Preparation~~ Example 1) was added to a slurry of 60% sodium hydride (1.75 g, 43.80 mmol) in DMSO (100 mL). This mixture was stirred until the solids dissolved (~60 min) and 2-bromo-1-(2-methyl-1H-imidazol-4-yl)-ethanone, hydrobromide (~~Preparation 10~~ Example 32) (3.50 g, 12.33 mmol) was added and the reaction was stirred 1 h at rt. Additional portions of sodium hydride (0.40g, 10.0 mmol) and 2-bromo-1-(2-methyl-1H-imidazol-4-yl)-ethanone, hydrobromide (1.25 g, 4.40 mmol) were added and after stirring 1hr more, the mixture was

poured into water (3 L). The pink precipitate was collected on a Celite pad and the material was then removed by rinsing with MeOH. Concentration of the rinse gave a yellow-orange solid that was partially purified by flash chromatography (10-20% MeOH/EtOAc as eluent) to give 2.18 g of a ~4:1 mixture of 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-{2-methyl-1-[2-(2-methyl-1H-imidazol-4-yl)-2-oxo-ethyl]-1H-imidazol-4-yl}-ethanone and 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2-methyl-1H-imidazol-4-yl)-ethanone (Example 2R) as an orange foam.

A portion (0.5 g) of this mixture was treated with triethylamine (0.4 mL, 2.87 mmol) and p-toluenesulfonyl chloride (0.25 g, 1.31 mmol) in CH_2Cl_2 (25 mL) and stirred overnight. The reaction was washed with water, dried ($MgSO_4$) and concentrated onto silica gel. Flash chromatography using a 5%-20% MeOH/EtOAc gradient elution gave first 0.19 g of 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-[2-methyl-1-(toluene-4-sulfonyl)-1H-imidazol-4-yl]-ethanone and then 0.31 g of N-tosylated 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-{2-methyl-1-[2-(2-methyl-1H-imidazol-4-yl)-2-oxo-ethyl]-1H-imidazol-4-yl}-ethanone.

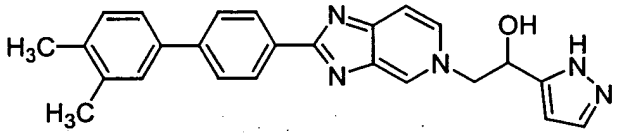
Saturation of a solution of 0.2 g of N-tosylated 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-{2-methyl-1-[2-(2-methyl-1H-imidazol-4-yl)-2-oxo-ethyl]-1H-imidazol-4-yl}-ethanone in ethanol (5 mL) with HCl gas followed by 2h stirring gave 0.137 g of the hydrochloride salt of 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-{2-methyl-1-[2-(2-methyl-1H-imidazol-4-yl)-2-oxo-ethyl]-1H-imidazol-4-yl}-ethanone as a tan-yellow which had: mp = 243-248°C (decomposes); H-NMR (DMSO-d_6) δ 9.50 (s, 1H), 8.69 (s, 1H), 8.62 (dd, J= 6.6, 1.2 Hz, 1H), 8.44 (d, J= 8.3 Hz, 2H), 8.25 (s, 1H), 8.22 (d, J= 6.6 Hz, 1H), 7.90 (d, J = 8.7 Hz, 2H), 7.57 (s, 1H), 7.49 (dd, J = 7.5, 1.9 Hz, 1H), 7.22 (d, J= 8.3 Hz, 1H), 6.28 (s, 2H), 5.79 (s, 2H), 2.60 (s, 3H), 2.39 (s, 3H), 2.27 (s, 3H), 2.23 (s, 3H). Anal. Calculated for $C_{32}H_{29}N_7O_2 \bullet 4HCl \bullet 2.5H_2O$: C, 52.33; H, 5.21; N, 13.35. Found: C, 52.20; H, 5.35; N, 13.09.

Example 20: Synthesis of 2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2-methyl-1H-imidazol-4-yl)-ethanol, **(50)**



Sodium borohydride (0.015 g, 0.397 mmol) was added to 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-[2-methyl-1-(toluene-4-sulfonyl)-1H-imidazol-4-yl]-ethanone (Example 3R) (0.200 g, 0.347 mmol) in ethanol (15 mL) and stirred 18 h at rt. Water (5 mL) was added and the mixture was concentrated, then redissolved in ethanol (10 mL) and filtered to remove a white solid. Saturation of the filtrate solution with HCl gas produced a yellow precipitate which was filtered and recrystallized from water/ethanol to give 0.039 g (23%) of -[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2-methyl-1H-imidazol-4-yl)-ethanol hydrochloride as a light yellow solid which had: mp >260°C; NMR (DMSO-d_6) δ 9.51 (br s, 1H), 8.61 (d, J= 7.1 Hz, 1H), 8.40 (br d, J = 7.5 Hz, 2H), 8.18 (br d, J= 6.2 Hz, 1H), 7.91 (d, J = 8.7 Hz, 2H), 7.56 (s, 1H), 7.49 (d, J = 7.9 Hz, 1H), 7.25 (s, 1H), 7.23 (d, J = 8.3 Hz, 1H), 6.64 (d, J = 5.4 Hz, 1H), .19-5.23 (m, 1H), 5.09-5.12 (br d, J = 12.9 Hz, 1H), 4.82 (dd, J= 12.9, 8.7 Hz, 1H), 2.53 (s, 3H), 2.28 (s, 3H), 2.23 (s, 3H).

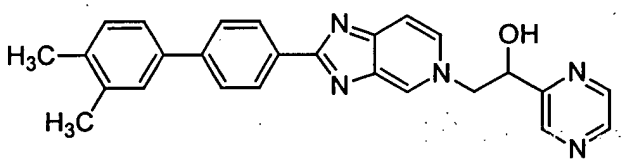
Example 21: Synthesis of 2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanol, **(51)**



Sodium borohydride (0.015 g, 0.40 mmol) was added to 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone (Example 3S) (0.12 g, 0.29 mmol) in ethanol (15 mL) and then stirred 2hr at rt. Water was added to precipitate a yellow solid which was collected and dried to give 0.087 g (73%) of 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanol. The maleate salt (EtOAc/EtOH) of this material had: mp ~ 230-245°C; NMR (DMSO-d_6) δ 9.42 (s, 1H), 8.61 (d, J = 6.6 Hz, 1H), 8.34 (d, J = 8.3 Hz, 2H), 8.15 (d, J = 6.6 Hz, 1H), 7.93 (d, J = 8.3 Hz, 2H), 7.69 (s, 1H), 7.59 (s, 1H), 7.51 (d, J = 7.5 Hz, 1H), 7.26 (d, J= 7.9 Hz, 1H), 6.27 (s, 1H), 6.03 (s, 2H), 5.91 (br s, 1H), 5.11 (br s, 1H), 5.02 (d, J = 12.9 Hz, 1H), 4.85-4.79 (m, 1H), 2.30 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.79, 160.49, 144.28, 138.22, 137.69, 137.41, 136.82, 135.98, 130.89,

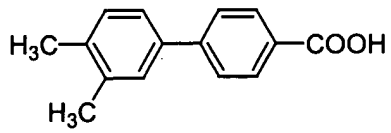
129.05, 128.50, 127.80, 127.14, 124.83, 111.37, 103.33, 67.45, 65.16, 20.19, 19.80; Anal. Calculated for $C_{25}H_{23}N_5O \bullet C_4H_4O_4 \bullet 0.5H_2O$: C, 65.16; H, 5.28; N, 13.10. Found: C, 64.79; H, 5.27; N, 12.84.

Example 22: Synthesis of 2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-pyrazin-2-yl-ethanol, **(52)**



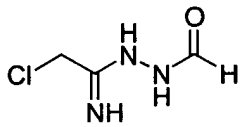
2-(3',4'-Dimethyl-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine hydrochloride (0.150 g, 0.447 mmol, ~~title compound from~~ Example 1) was added to a slurry of 60% sodium hydride (0.055 g, 1.375 mmol) in DMSO (10 mL). This mixture was stirred until the solids dissolved (~15 min) and 2-bromo-1-pyrazin-2-yl-ethanone hydrobromide (~~Preparation 17~~ Example 39) (0.151 g, 0.536 mmol) was added to give a burgundy red solution. The reaction was stirred 18 h at rt, concentrated and loaded onto silica gel. Flash chromatography with 5-10% methanol/ethyl acetate gave 0.199 g of crude 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-pyrazin-2-yl-ethanone as an orange solid. This was dissolved in 10 mL 20:1 ethyl acetate/ methanol, leaving behind an insoluble pinkish tan impurity. The solution was concentrated, redissolved in ethanol and treated with sodium borohydride (0.050 g, 1.32 mmol) and stirred for 2 h. The mixture was diluted with water and the tan colored precipitate was collected to yield 0.058 g (31%) of 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-pyrazin-2-yl-ethanol, the maleate salt of which had: mp ~230°C (dec.); NMR (DMSO-d_6) δ 9.43 (s, 1H), 8.77 (d, J= 1.2 Hz, 1H), 8.68-8.64 (m, 2H), 8.57 (d, J = 7.5 Hz, 1H), 8.34 (d, J = 8.7 Hz, 2H), 8.13 (d, J = 6.6 Hz, 1H), 7.92 (d, J = 8.7Hz, 2H), 7.58 (s, 1H), 7.50 (dd, J = 7.9, 1.6 Hz, 1H), 7.25 (d, J = 8.3 Hz, 1H), 6.39 (d, J = 5.8 Hz, 1H), 6.01 (s, 2H), 5.28-5.22 (m , 1H), 5.13 (dd, J = 13.3, 3.3 Hz, 1H), 4.87 (dd, J = 13.3, 8.7 Hz, 1H), 2.30 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.79, 160.67, 155.73, 145.07, 144.41, 144.31, 144.05, 138.24, 137.69, 137.42, 136.81, 135.84, 130.89, 129.07, 128.50, 127.81, 127.16, 124.83, 114.46, 71.56, 64.57, 20.19, 19.79; Anal. Calculated for $C_{26}H_{23}N_5O \bullet C_4H_4O_4$: C, 67.03; H, 5.06; N, 13.03. Found: C, 66.66; H, 5.08; N, 12.85.

Example 23: Synthesis of 3',4'-Dimethyl-biphenyl-4-carboxylic acid, **(53)**



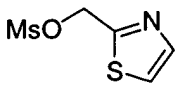
3,4 – Dimethylphenylboronic acid (6.4 g, 42.7 mmol), 4-bromobenzoic acid (10.5g, 52.7 mmol) sodium carbonate (13.6 g, 128.3 mmol) and tetrakis(triphenylphosphine)palladium (0) (1.2 g, 1.0 mmol) in dimethoxyethane (90 mL)/ water (30 mL) were refluxed for 5 h then concentrated. The residue was carefully treated with 1N HCl and extracted into ethyl acetate. The extract was washed with brine, dried ($MgSO_4$) and concentrated to a light orange solid (11.4 g). Recrystallization from ethanol gave 7.4 g (77% - 2 crops) of ~95% pure 3',4'-dimethyl-biphenyl-4-carboxylic acid as a white solid which had: mp 211-213 ° C; NMR (~~$CDCl_3$~~ DMSO-d_6) δ 12.94 (s, 1H), 7.97 (d, J = 8.3 Hz, 2H), 7.74 (d, J = 8.3 Hz, 2H), 7.51 (d, J = 1.2 Hz, 1H), 7.43 (dd, J = 7.9, 2.1 Hz, 1H), 7.23 (d, J = 7.9 Hz, 1H), 2.28 (s, 3H), 2.24 (s, 3H)

Example 24: Synthesis of Formic acid N'-(2-chloro-1-imino-ethyl)-hydrazide, **(54)**



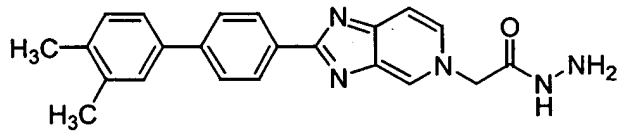
Sodium methoxide (0.032 g, 0.592 mmol) was added to an ice cold solution of chloroacetonitrile (1.26 mL, 19.9 mmol) in methanol (15 mL). After stirring for 45 min, acetic acid (0.034 mL, 0.594 mmol) was added to neutralize the methoxide and formylhydrazine (1.18 g, 19.6 mmol) was added. The mixture was stirred at rt for an additional hour and concentrated to a yellow solid. Trituration with ethanol (20 mL) gave 1.15 g (43%) of formic acid N'-(2-chloro-1-imino-ethyl)-hydrazide as a white solid which NMR (DMSO-d_6) showed to be a mixture of hydrated and non-hydrated formamides which was used without further purification.

Example 25: Synthesis of Methanesulfonic acid thiazol-2-ylmethyl ester, **(55)**



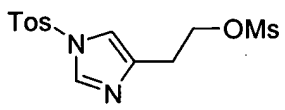
Sodium borohydride (0.17 g, 4.49 mmol) was added to a solution of 2-thiazolecarboxaldehyde (0.39 mL, 4.44 mmol) in ethanol (25 mL). After stirring 1.5 h at rt, the reaction was quenched with water and concentrated. The residue was dissolved in ethyl acetate and washed with water and brine, dried over magnesium sulfate and concentrated to give 0.27 g, (53%) of thiazol-2-yl-methanol as a tan oil which had: NMR ($CDCl_3$) δ 7.72 (d, J = 3.3 Hz, 1H), 7.31 (d, J = 3.3 Hz, 1H), 4.95 9 (s, 2H), 2.83 (br s, 1H). This was mixed with triethyl amine (0.33 mL, 2.37 mmol) in methylene chloride (4 mL) and cooled in ice. Methanesulfonylchloride (0.18 mL, 2.33 mmol) in methylene chloride (2mL) was added dropwise over 1 min. After 30 min stirring at 0°C, the reaction was washed with water and brine, dried over magnesium sulfate and concentrated to yield 0.41 g (91%) of methanesulfonic acid thiazol-2-ylmethyl ester as an orange oil which had: NMR ($CDCl_3$) δ 7.83 (d, J = 3.3 Hz, 1H), 7.45 (d, J = 3.3 Hz, 1H), 5.50 (s, 2H), 3.06 (s, 3H).

Example 26: Synthesis of 2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetic acid hydrazide, **(56)**



[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetic acid methyl ester (0.40 g, 1.08 mmol, Example 3A) and hydrazine hydrate (0.55 mL, 11.3 mmol) in ethanol (15 mL) were refluxed for 16h. The white precipitate that formed was collected and rinsed with 3mL ethanol to yield 0.290 g (73%) of 85-90% pure 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetic acid hydrazide which had NMR (DMSO-d_6) δ 8,78 (d, J = 1.2 Hz, 1H), 8.40 (d, J = 8.7 Hz, 2H), 7.92 (dd, J = 6.6, 1.5 Hz, 1H), 7.73 (d, J = 8.3 Hz, 2H), 7.68 (d, J = 6.6 Hz, 1H), 7.52 (s, 1H), 7.44 (dd, J = 7.9, 1.7 Hz, 1H), 7.21 (d, J = 7.9 Hz, 1H), 5.09 (s, 2H), 4.38 (br s, 2H), 2.29 (s, 3H), 2.24 (s, 3H).

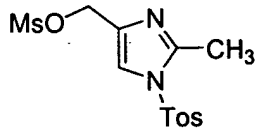
Example 27: Synthesis of Methanesulfonic acid 2-[1-(toluene-4-sulfonyl)-1H-imidazol-4-yl]-ethyl ester, **(57)**



4-Imidazoleacetic acid hydrochloride (5.93 g, 36.47 mmol) in methanol (125 mL) was saturated with HCl gas and stirred at rt for5.5 h then concentrated to give a quantitative yield of 4-imidazoleacetic acid, methyl ester, hydrochloride as a white solid which had NMR (MeOH- d_4) δ 8.87 (d, J = 1.2 Hz, 1H), 7.46 (s, 1H), 3.89 (s, 2H), 3.72 (s, 3H). This was added portionwise to a slurry of lithium aluminum hydride (2.8 g, 74.0 mmol) in THF (150 mL). The mixture was refluxed for 3h, cooled in ice and carefully quenched with excess sodium sulfate decahydrate. This slurry was dried with anh. sodium sulfate and filtered through Celite with ethyl acetate rinse. Concentration gave 2.6 g of a colorless oil that NMR ($CDCl_3$) showed to be a 1.5:1 mixture of starting ester and 2-(1H-imidazol-4-yl)-ethanol. This crude mixture was slurried in methylene chloride (30 mL) and triethylamine (3.3 mL, 23.6 mmoL) and p-toluenesulfonylchloride (4.45 g, 23.3 mmol) was added. THF (30 mL) was added to help dissolve the starting material. A vigorous reaction ensued. After stirring 20 min, the reaction was concentrated, the residue was partitioned between ethyl acetate and water. The organics were washed with sat. $NaHCO_3$ solution and brine, dried ($MgSO_4$) and concentrated to an oily white solid. Ether (50 mL) was added and the mixture was stirred vigorously for 30 min. to break up the solid. This material was filtered off and rinsed with ether to give 2.96g (30% from 4-imidazoleacetic acid hydrochloride) of pure 2-[1-(toluene-4-sulfonyl)-1H-imidazol-4-yl]-ethanol as a white solid which had: NMR ($CDCl_3$) δ 7.93 (d, J = 1.2 Hz, 1H), 7.79 (d, J = 8.3 Hz, 2H), 7.33 (d, J= 8.3 Hz, 2H), 7.05 (d, J = 1.2 Hz, 1H), 3.83 (t, J = 5.8 Hz, 2H), 2.74-2.71 (m, 2H), 2.42 (s, 3H).

Methanesulfonyl chloride (0.29 mL, 3.75 mmol) in methylene chloride (5 mL) was added dropwise over 1-2 min to an ice cold slurry of 2-[1-(toluene-4-sulfonyl)-1H-imidazol-4-yl]-ethanol (1.00 g, 3.75 mmol) and triethylamine (0.53 mL, 3.80 mmol) in methylene chloride (20 mL). The solids dissolved to give a light yellow solution which was stirred 1.25h at 0°C then washed with water, dried ($MgSO_4$) and concentrated to give 1.1 g (85%) of methanesulfonic acid 2-[1-(toluene-4-sulfonyl)-1H-imidazol-4-yl]-ethyl ester as a straw colored oil which upon evacuation slowly solidified to a waxy, light yellow solid which had: NMR ($CDCl_3$) δ 7.92 (s, 1H), 7.79 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.3 Hz, 2H), 7.11 (d, J = 1.2 Hz, 1H), 4,42 (t, J = 6.6 Hz, 2H), 2.94 (t, J = 6.6 Hz, 2H), 2.86 (s, 3H), 2.42 (s, 3H).

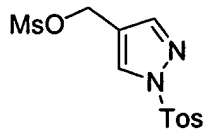
Example 28: Synthesis of Methanesulfonic acid 2-methyl-1-(toluene-4-sulfonyl)-1H-imidazol-4-ylmethyl ester, **(58)**



2-Methyl-3H-imidazole-4-carbaldehyde (2.00 g, 18.16 mmol), triethylamine (3.0 mL, 21.5 mmol) and p-toluenesulfonylchloride (3.5 g, 18.36 mmol) were combined in methylene chloride (50 mL) and stirred at rt for 16 h. The reaction was concentrated and the residue partitioned between ethyl acetate and water, the organics then washed with brine, dried ($MgSO_4$) and concentrated to give 4.1 g (85%) of 2-methyl-1-(toluene-4-sulfonyl)-1H-imidazole-4-carbaldehyde as a yellow solid which had : NMR ($CDCl_3$) δ 9.79 (s, 1H), 8.04 (s, 1H), 7.79 (d, J = 8.7 Hz, 2H), 7.38 (d, J = 8.7 Hz, 2H), 2.53 (s, 3H), 2.44 (s, 3H). This material was dissolved in ethanol (50 mL) and sodium borohydride (0.30 g, 7.93 mmol) was added. After stirring 2 h at rt, water was added and the mixture was concentrated. The residue was partitioned between ethyl acetate and water, the organics then washed with brine, dried ($MgSO_4$) and concentrated to give 1.67g (83%) of [2-methyl-1-(toluene-4-sulfonyl)-1H-imidazol-4-yl]-methanol as a white solid which had: NMR ($CDCl_3$) δ 7.75 (d, J = 8.3 Hz, 2H), 7.34 – 7.30 (m, 3H), 4.48 (s, 2H), 2.48 (s, 3H), 2.42 (s, 3H).

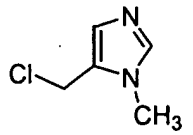
[2-Methyl-1-(toluene-4-sulfonyl)-1H-imidazol-4-yl]-methanol (0.50 g, 1.88 mmol) and triethylamine (0.31 mL, 2.22 mmol) in methylene chloride (22 mL) were cooled in an ice bath and methanesulfonylchloride (0.155 mL, 2.00 mmol) in methylene chloride (3 mL) was added dropwise over 1 min. The ice bath was removed and the reaction was stirred at rt for 1h and then concentrated. The residue was partitioned between ethyl acetate and water, the organics then washed with brine, dried ($MgSO_4$) and concentrated to give 0.61 g (94%) of methanesulfonic acid 2-methyl-1-(toluene-4-sulfonyl)-1H-imidazol-4-ylmethyl ester as a white solid which had: NMR ($CDCl_3$) δ 7.77 (d, J = 8.3 Hz, 2H), 7.51 (s, 1H), 7.36 (d, J = 8.3 Hz, 2H), 5.07 (s, 2H), 3.00 (s, 3H), 2.50 (s, 3H), 2.44 (s, 3H).

Example 29: Synthesis of Methanesulfonic acid 1-(toluene-4-sulfonyl)-1H-pyrazol-4-ylmethyl ester, **(59)**



1H-Pyrazole-4-carboxylic acid ethyl ester (0.50 g, 3.57 mmol), triethylamine (0.55 mL, 3.95 mmol) and p-toluenesulfonylchloride (0.71 g, 3.72 mmol) were combined in methylene chloride (20 mL) and stirred for 1h at rt. The mixture was concentrated and the residue partitioned between ethyl acetate and water, the organics were washed with brine, dried ($MgSO_4$) and concentrated to give 1.05 g of 1-(toluene-4-sulfonyl)-1H-pyrazole-4-carboxylic acid ethyl ester as an oily white solid. Lithium borohydride (0.10 g, 4.59 mmol) was added to a slurry of this material (0.80 g, 2.72 mmol) and the mixture was stirred at rt for 16h. An additional portion of lithium borohydride (0.10 g) was added at 16 h, and another 0.15 g was added at 40 h with methanol (15 mL) to help dissolve the unreacted starting material. After 64h total reaction time, the mixture was quenched with water and concentrated, the residue was partitioned between ethyl acetate and water, the organics were washed with brine, dried ($MgSO_4$) and concentrated to give 0.46 g of a 1:1 mixture of starting ester and [1-(toluene-4-sulfonyl)-1H-pyrazol-4-yl]-methanol as a colorless oil. Flash chromatography using a 20-50% ethyl acetate/ hexanes gradient afforded 0.149 g (21%) of pure alcohol as a white solid which had: NMR ($CDCl_3$) δ 8.07 (s, 1H), 7.90 (d, J= 8.3 Hz, 2H), 7.73 (s, 1H), 7.33 (d, J = 8.3 Hz, 2H), 4.61 (s, 2H), 2.43 (s, 3H). This material was combined with triethyl amine (0.10 mL, 0.72 mmol) in methylene chloride (8 mL), cooled in ice and methanesulfonylchloride (0.046 mL, 0.594 mmol) in methylene chloride (2 mL) was added. After stirring 16h at rt, the reaction was concentrated, the residue was partitioned between ethyl acetate and water, the organics were washed with brine, dried ($MgSO_4$) and concentrated to give a 0.13 g of a ~ 1:1:0.5 mixture of [methanesulfonic acid 1-(toluene-4-sulfonyl)-1H-pyrazol-4-ylmethyl ester, 4-chloromethyl-1-(toluene-4-sulfonyl)-1H-pyrazole and starting alcohol. This mixture was used immediately without purification.

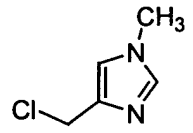
Example 30: Synthesis of 5-Chloromethyl-1-methyl-1H-imidazole hydrochloride, **(60)**



3-Methyl-3H-imidazole-4-carboxylic acid ethyl ester (*Chem. Pharm. Bull*, **1994**, *42*, 1463) (0.62 g, 4.02 mmol) in THF (5mL) was added dropwise over 1 min. to a slurry of lithium aluminum hydride (0.25 g, 6.60 mmol) in THF (20 mL). After stirring 16h at rt, the reaction was carefully quenched with excess sodium sulfate decahydrate, dried with anh. sodium sulfate and filtered through Celite. Concentration gave 0.33 g (73%) of (3-methyl-3H-imidazol-4-yl)-methanol as a white solid which had: NMR ($CDCl_3$) δ 7.38 (s, 1H), 6.87 (s, 1H), 4.60 (s, 2H), 3.69 (s, 3H). A solution of this alcohol (0.150 g, 1.34 mmol) in thionyl chloride (5 mL) was refluxed for 3 h and concentrated. The residue was dissolved in a minimum of ethanol and ether was added to precipitate a white solid. This was collected to yield 0.185 g (83%) of 5-chloromethyl-1-methyl-1H-imidazole hydrochloride which had: NMR (DMSO-d_6) δ 9.15 (s, 1H), 7.75 (d, J = 1.2 Hz, 1H), 4.99 (s, 2H), 3.84 (s, 3H).

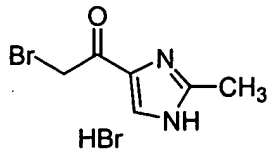
Example 31: Synthesis of ~~T~~the compound below was made by essentially the same procedure as exemplified in ~~Preparation 8~~ Example 30:

4-Chloromethyl-1-methyl-1H-imidazole hydrochloride, **(61)**



Prepared from 1-methyl-1H-imidazole-4-carboxylic acid ethyl ester (*Chem. Pharm. Bull*, **1994**, *42*, 1463). Obtained as a sticky orange solid and used without purification.

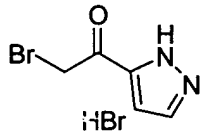
Example 32: Synthesis of 2-Bromo-1-(2-methyl-1H-imidazol-4-yl)-ethanone, hydrobromide, **(62)**



Bromine (2.1 mL, 40.98 mmol) was added dropwise over 5 min to a ~90-95°C solution of 1-(2-methyl-1H-imidazol-4-yl)-ethanone (*Tetrahedron.Lett,* **1985**, *26*, 3423) (5.00 g, 40.28 mmol) in 48% aq. HBr (100 mL). After 30 min stirring, an additional 0.2 mL (3.90 mmol) of bromine was added and stirring continued for another 30 min. The reaction was concentrated, triturated with acetone, filtered and dried under N_2 to give 9.74 g (85%) of 2-bromo-1-(2-methyl-1H-imidazol-4-yl)-ethanone, hydrobromide salt as a light tan solid: H-NMR (DMSO-d_6) δ 8.57 (s, 1H), 4.69 (s, 2H), 2.54 (s, 3H).

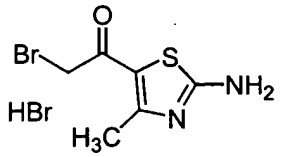
Example ~~2~~33: The compounds below were all made by essentially the same procedure as exemplified in ~~Preparation 10~~ Example 32.

A. 2-Bromo-1-(2H-pyrazol-3-yl)-ethanone, **(63)**



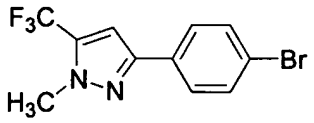
Prepared from 1-(2H-pyrazol-3-yl)-ethanone– yield 26%. H-NMR (DMSO-d_6) δ 7.87 (d, J = 2.5 Hz, 1H), 6.79 (d, J = 2.5 Hz, 1H), 4.71 (s, 2H).

B. 1-(2-Amino-4-methyl-thiazol-5-yl)-2-bromo-ethanone, **(64)**



Prepared from 1-(2-amino-4-methyl-thiazol-5-yl)-ethanone– yield 70%. H-NMR (MeOH-d_4) δ 4.87 (br s, 3H), 4.42 (s, 2H), 2.59 (s, 3H).

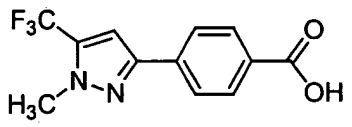
Example 34: Synthesis of 3-(4-Bromo-phenyl)-1-methyl-5-trifluoromethyl-1H-pyrazole, **(65)**



Potassium t-butoxide (84.6 g, 753.5 mmol) was added in 3 portions over 5 min to a solution of 4-bromoacetophenone (30.0 g, 150.7 mmol) in THF (500 mL). The mixture was cooled in a water bath and ethyl trifluoroacetate (89.7 mL, 753.5 mmol) was added dropwise over 5min. 18-Crown-6 (7.97 g, 30.1 mmol) was added and the resulting mixture was stirred at rt for 4.5 hrs. The mixture was poured into water (1L), extracted with ethyl acetate (3 X500 mL), dried over sodium sulfate and concentrated. An additional reconcentration from toluene to remove residual water gave 1-(4-bromo-phenyl)-4,4,4-trifluoro-3-hydroxy-but-2-en-1-one as a yellow oil which had: NMR ($CDCl_3$) δ 7.81 (d, J = 8.7 Hz, 2H), 7.66 (d, J = 9.1 Hz, 2H), 6.55-6.54 (m, 1H). This was dissolved in toluene (500 mL) and hydrazine monohydrate (50 mL) was added dropwise over 5 min. The mixture was heated to 95°C for 14h, cooled and concentrated to a yellow-orange solid. Hot hexanes (300 mL) was added and the mixture allowed to recool to rt. The resulting white precipitate was collected to afford 27.5 g of 3-(4-3-bromo-phenyl)-5-trifluoromethyl-1H-pyrazole. Concentration of the mother liquors and trituration with hexanes yielded another 9.4 g of material (total yield = 84%). NMR ($CDCl_3$) δ 7.61 (d, J = 8.3 Hz, 2H) 7.44 (d, J = 8.7 Hz, 2H), 6.79 (s, 1H).

A mixture of dimethylsulfate (35.7 mL, 377.5 mmol) and 3-(4-3-bromo-phenyl)-5-trifluoromethyl-1H-pyrazole (27.47 g, 94.38 mmol) in toluene (700 mL) was heated for 12.5h at 100°C, then cooled and poured into 1N NaOH (1L). This was extracted with ethyl acetate (3 X 500 mL) and the extracts were concentrated. Flash chromatography on silica gel using 5-10% ethyl acetate/ hexanes as eluent followed by evacuation on a vacuum pump for ~60 hrs to remove the residual dimethylsulfate gave 27.56 g (96%) of 3-(4-bromo-phenyl)-1-methyl-5-trifluoromethyl-1H-pyrazole as a white solid which had: NMR ($CDCl_3$) δ 7.63 (d, J = 8.7 Hz, 2H), 7.53 (d, J = 8.7 Hz, 2H), 6.87 (s, 1H), 4.02 (s, 3H).

Example 35: Synthesis of 4-(1-Methyl-5-trifluoromethyl-1H-pyrazol-3-yl)-benzoic acid, **(66)**

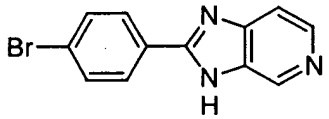


t-Butyllithium (1.7 M in pentane, 6.75 mL, 11.48 mmol) was added to a –78°C solution of 3-(4-bromo-phenyl)-1-methyl-5-trifluoromethyl-1H-pyrazole (~~Preparation 12~~ Example 34) (3.48 g, 11.41 mmol) in THF (50 mL) to give an orange-brown solution. After stirring for 3-5 min, carbon dioxide has bubbled in for ~ 3 min to give an orange solution. This was stirred 15 min at –78°C and then 1h at ambient temperature. Saturated ammonium chloride was added, and the reaction was concentrated. The material was dissolved in 1N sodium hydroxide and washed with ethyl acetate. The aqueous layer was acidified with 1N HCl to ~ pH 4 and extracted into ethyl acetate, dried ($MgSO_4$) and concentrated to give 4-(1-methyl-5-trifluoromethyl-1H-pyrazol-3-yl)-benzoic acid as a white solid (2.19g, 71%) which had: mp 183-185°C; NMR ($CDCl_3$) δ 8.13 (d, J = 8.7 Hz, 2H), 7.85 (d, J = 8.7 Hz, 2H), 6.95 (s, 1H), 4.04 (s, 3H).

Example 36: ~~Synthesis of These compounds were made by essentially the same procedure as exemplified in~~ The compounds below were all made by essentially the same procedure as exemplified in Example 1

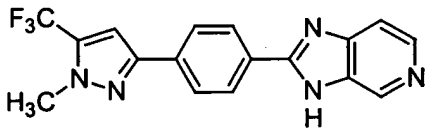
Example 1

A. 2-(4-Bromo-phenyl)-3H-imidazo[4,5-c]pyridine, **(67)**



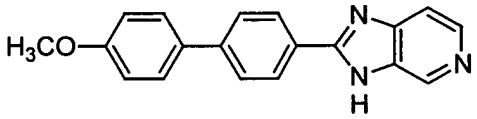
Using 4-bromobenzoic acid –yield 80%, free base had: mp > 260°C; NMR (DMSO-d_6) δ 8.83 (s, 1H), 8.17-8.06 (m, 3H), 7.68 (d, J = 8.3 Hz, 2H), 7.45 (d, J = 5.4 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 155.39, 145.18, 140.82, 139.61, 132.52, 131.06, 129.52, 124.03, 110.23.

B. 2-[4-(1-Methyl-5-trifluoromethyl-1H-pyrazol-3-yl)-phenyl]-3H-imidazo[4,5-c]pyridine hydrochloride, **(68)**



Using 4-(1-methyl-5-trifluoromethyl-1H-pyrazol-3-yl)-benzoic acid (~~see preparation 13~~ Example 35) and keeping the temperature between 165-170°C for ~5 h – yield 39% of a white solid which had: mp > 260°C; MS: M^+ 344; NMR (DMSO-d_6) δ 9.41 (s, 1H), 8.54 (d, J = 6.6 Hz, 1H), 8.38 (d, J = 8.3 Hz, 2H), 8.12-8.09 (m, 3H), 7.57 (s, 1H), 4.02 (s, 3H).

Example 37: Synthesis of 2-(4'-Methoxy-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine, **(69)**

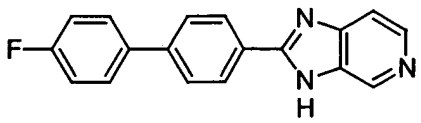


2-(4-Bromo-phenyl)-3H-imidazo[4,5-c]pyridine (~~Preparation 14A~~ Example 36A) (0.25 g, 0.912 mmol), 4-methoxyphenylboronic acid (0.20 g, 1.32 mmol), tetrakis(triphenylphosphine)palladium (0) and potassium carbonate (0.126 g, 2.32 mmol) in ethanol/water (13 mL/1.5 mL) were refluxed for 2.5h. The mixture was diluted with ethanol (20 mL) and water (20 mL), filtered (Celite) and concentrated. The residue was triturated with saturated aq. $NaHCO_3$ (20 mL), water and ether (50 mL) to give 0.26 g (95%) of 2-(4'-methoxy-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine as a light pink-tan tinted solid which had: mp > 260°C; NMR (DMSO-d_6) δ 8.90 (s, 1H), 8.26 (d, J = 5.4 Hz, 1H), 8.22 (d, J = 8.7 Hz, 2H), 7.81

(d, J = 8.7 Hz, 2H), 7.70 (d, J = 9.1 Hz, 2H), 7.55 (d, J = 5.4 Hz, 1H), 7.02 (J = 8.7 Hz, 2H), 3.77 (s, 3H).

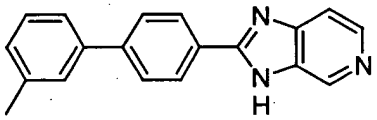
Example 38: The compounds below were made by essentially the same procedure as exemplified in Example 37.

A. 2-(4'-Fluoro-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine, **(70)**



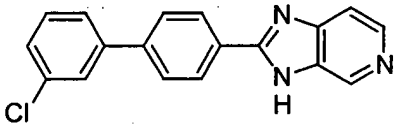
Using 4-fluorophenylboronic acid – yield 80% as a tan solid which had: mp > 260°C; NMR (DMSO-d_6) δ 8.85 (d, J = 0.8 Hz, 1H), 8.27 (d, J = 8.3 Hz, 2H), 8.18 (d, J = 5.4 Hz, 1H), 7.82-7.77 (m, 4H), 7.50 (dd, J = 5.4, 0.8 Hz, 1H), 7.29 (t, J = 8.7 Hz, 2H).

B. 2-(3'-Methyl-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine, **(71)**



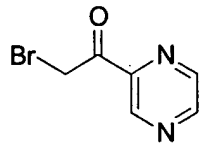
Using 3-tolylboronic acid: mp 209-211°C; NMR (DMSO-d_6) δ 13.36 (br s, 1H), 8.91 (s, 1H), 8.28-8.24 (m, 3H), 7.83 (d, J = 7.5 Hz, 2H), 7.55 (s, 2H), 7.51 (d, J = 7.9 Hz, 1H), 7.33 (t, J = 7.5 Hz, 1H), 7.16 (d, J = 7.5 Hz, 1H), 7.16 (d, J = 7.5 Hz, 1H), 2.34 (s, 3H).

C. 2-(3'-Chloro-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine, **(72)**



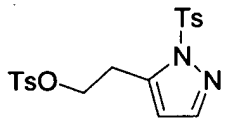
Using 3-chlorophenylboronic acid: mp 250-252°C; NMR (DMSO-d_6) δ 8.87 (s, 1H), 8.29 (d, J = 8.0 Hz, 2H), 8.21 (d, J = 5.0 Hz, 1H), 7.85 (d, J = 7.9 Hz, 2H), 7.79 (s, 1H), 7.69 (d, J = 7.5 Hz, 1H), 7.58-7.40 (m 4H).

Example 39: Synthesis of 2-Bromo-1-pyrazin-2-yl-ethanone hydrobromide, **(73)**



Bromine (0.23 mL, 4.49 mmol) was added over 3 min. to a ~80°C solution of 2-acetylpyrazine (0.50 g, 4.09 mmol) in 48% HBr (10 mL). The resulting mixture was heated between 80-90°C for 1h, concentrated then reconcentrated again from acetone. Trituration of the resulting red-brown solid with ether/acetone (20 mL/5 mL) gave 0.94 g (82%) of 2-bromo-1-pyrazin-2-yl-ethanone as a hydrobromide salt which had: NMR (DMSO-d_6) 9.12 (d, 1.2 Hz, 1H), 8.89 (d, J = 2.5 Hz, 1H), 8.78 (dd, J = 2.5, 1.7 Hz, 1H), 8.07 br s, 1H + residual water), 4.96 (s, 2H).

Example 40: Synthesis of Toluene-4-sulfonic acid 2-[2-(toluene-4-sulfonyl)-2H-pyrazol-3-yl]-ethyl ester, **(74)**



(2H-Pyrazol-3-yl)-acetic acid ethyl ester (JACS, 75, 1953, 4048) (0.94 g, 6.10 mmol) in 50 mL THF was added dropwise over 20 min to a slurry of LAH in 50 mL THF. The resulting mixture was stirred at ambient temperature overnight then refluxed for 7 h. Following quenching with sodium sulfate decahydrate and drying over anh. sodium sulfate, the reaction was filtered (Celite) and concentrated to give 0.49 g (72%) of 2-(2H-pyrazol-3-yl)-ethanol as a colorless oil: NMR ($CDCl_3$) δ 7.49 (d, J = 2.1 Hz, 1H), 6.13 (d, J = 2.1 Hz, 1H), 3.90 (t, J = 5.8 Hz, 2H), 2.91 (t, J = 5.8 Hz, 2H).

p-Toluenesulfonyl chloride (0.67 g, 3.51 mmol) was added to an ice cold solution of 2-(1H-pyrrol-2-yl)-ethanol (0.39 g, 3.48 mmol) and triethylamine (0.98 mL, 7.03 mmol) in methylene chloride (20 mL). After stirring for 2h, the reaction was partitioned between ethyl acetate and water. The organics were washed with water and brine, dried ($MgSO_4$) and concentrated to a

yellow oil. Chromatography on silica gel with 50% ethyl acetate/hexanes gave 0.284 g (19%) of the ditosylate, toluene-4-sulfonic acid 2-[2-(toluene-4-sulfonyl)-2H-pyrazol-3-yl]-ethyl ester, as a colorless oil: NMR ($CDCl_3$) δ 7.93 (d, J = 2.8 Hz, 1H), 7.80 (d, J = 8.7 Hz, 2H), 7.68 (d, J = 8.7 Hz, 2H), 7.28 (d, J= 8.3 Hz, 4H), 6.20 (d, J = 2.5 Hz, 1H), 4.20 (t, J = 6.8 Hz, 2H), 2.94 (t, J = 6.6 Hz, 2H), 2.42 (s, 3H), 2.39 (s, 3H).

Example 41: Protocol for the C3a Receptor Binding Assay:

The primary assay utilizes 125I labeled human C3a peptide binding to mouse B-cells (L1.2) which have been stably transfected with the human C3a receptor. Receptor and ligand were incubated in assay buffer for 45 minutes with shaking at room temperature in a 96-well plate format. A 250- fold excess of unlabelled human C3a peptide defined non-specific binding. The reaction was pelleted by centrifugation and terminated by filtration over glass fiber A filters with ice-cold wash buffer. Activity was counted on a Wallac beta scintillation counter. The compounds were tested for IC_{50} determination across a 3-log unit range. Table 1 shows IC50 data for the compounds of the present invention.

Table 1

Compound	IC_{50}
1	324 nM
2	225 nM
3	246 nM
4	67 nM
5	182 nM
6	160 nM
7	128 nM

8	630 nM
9	593 nM
10	260 nM
11	220 nM
12	410 nM
13	661 nM
14	622 nM
15	115 nM
16	366 nM
17	104 nM
18	213 nM
19	177 nM
20	39 nM
21	25 nM
22	55 nM

23	117 nM
24	91 nM
25	100 nM
26	168 nM
27	63 nM
28	98 nM
29	54 nM
30	186 nM
31	245 nM
32	117 nM
33	212 nM
34	398 nM
35	170 nM
36	452 nM
37	595 nM
38	167 nM

39	334 nM
40	306 nM
41	143 nM
42	78 nM
43	303 nM
44	121 nM
45	197 nM
46	81 nM
47	42 nM
48	112 nM
49	108 nM
50	144 nM
51	93 nM
52	241 nM

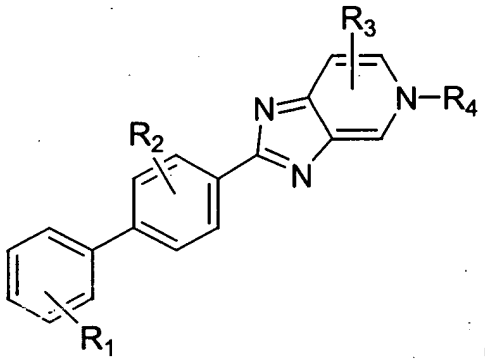
All of the references, patents, and publications cited herein are hereby incorporated by reference in their entirety.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the compounds and methods of use thereof described

herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.

What is claimed is:

1. A compound represented by Formula I



(I)

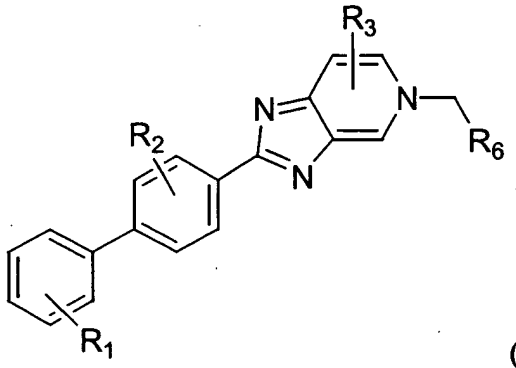
wherein,

R_1, R_2, R_3, and R_4,independently for each occurrence, represent one or more substituents selected from the group consisting of hydrogen, hydroxy, halo, amino, $C(=X)R_7$ (wherein X is NR_7, O or S), $-OC(=O)-R_7$, $-C(=O)O-R_7$, $-N(R_7)_2$, $-NR_7C(=O)-R_7$, $-C(=O)N(R_7)_2$, $-OC(=O)-N(R_7)_2$, $-NR_7-C(=O)-N(R_7)_2$, $-NR_7-C(NR_7)-N(R_7)_2$, $-P(O)_n-$ (wherein n is 0, 1, or 2), $-S(O)_n-$ (wherein n is 0, 1, or 2), $-S(O)_nN(R_7)_2$, (C_1-C_6)alkoxy-, (C_1-C_6)acyloxy-, (C_1-C_6)alkylamino-, $((C_1-C_6)alkyl)_2$amino-, (C_1-C_6)acylamino-, cyano, nitro, (un)substituted (C_1-C_6)alkyl-, (un)substituted (C_2-C_6)alkenyl-, (un)substituted (C_2-C_6)alkynyl-, (un)substituted (C_6-C_{10})aryl, (un)substituted (C_4-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl-, (un)substituted (C_2-C_{10})heterocycloalkyl-;

or R_3 and R_4, taken together form a (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_3-C_{10})heterocycloalkyl, (un)substituted (C_5-C_{10})aryl, or (un)substituted (C_4-C_{10})heteroaryl; and

R_7, independently for each occurrence, represents one or more of H, (un)substituted (C_1-C_{10})alkyl, (un)substituted (C_2-C_{10})alkenyl , (un)substituted (C_2-C_{10})alkynyl, (C_6-C_{10})aryl, (un)substituted (C_1-C_{10})heteroalkyl, (un)substituted (C_5-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_2-C_{10})heterocycloalkyl.

2. A compound represented by Formula II



(II)

wherein,

R_1, R_2, R_3, and R_6,independently for each occurrence, represent one or more substituents selected from the group consisting of hydrogen, hydroxy, halo, amino, $C(=X)R_7$ (wherein X is NR_7, O or S), $-OC(=O)-R_7$, $-C(=O)O-R_7$, $-N(R_7)_2$, $-NR_7C(=O)-R_7$, $-C(=O)N(R_7)_2$, $-OC(=O)-N(R_7)_2$, $-NR_7-C(=O)-N(R_7)_2$, $-NR_7-C(NR_7)-N(R_7)_2$, $-P(O)_n-$ (wherein n is 0, 1, or 2), $-S(O)_n-$ (wherein n is 0, 1, or 2), $-S(O)_nN(R_7)_2$, (C_1-C_6)alkoxy-, (C_1-C_6)acyloxy-, (C_1-C_6)alkylamino-, $((C_1-C_6)alkyl)_2$amino-, (C_1-C_6)acylamino-, cyano, nitro, (un)substituted (C_1-C_6)alkyl-, (un)substituted (C_2-C_6)alkenyl-, (un)substituted (C_2-C_6)alkynyl-, (un)substituted (C_6-C_{10})aryl, (un)substituted (C_4-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl-, (un)substituted (C_2-C_{10})heterocycloalkyl-;

or R_3 and R_4, taken together form a (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_3-C_{10})heterocycloalkyl, (un)substituted (C_5-C_{10})aryl, or (un)substituted (C_4-C_{10})heteroaryl; and

R_7, independently for each occurrence, represents one or more occurrences of H, (un)substituted (C_1-C_{10})alkyl, (un)substituted (C_2-C_{10})alkenyl , (un)substituted (C_2-C_{10})alkynyl, (C_6-C_{10})aryl, (un)substituted (C_1-C_{10})heteroalkyl, (un)substituted (C_5-C_{10})heteroaryl, (un)substituted (C_3-C_{10})cycloalkyl, (un)substituted (C_2-C_{10})heterocycloalkyl.

3. The compound of claim 1 selected from the group consisting of 2-(3',4'-dimethyl-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-methyl-5H-imidazo[4,5-c]pyridine; [2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetic acid methyl ester; [2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetonitrile; 5-allyl-2-(3',4'-dimethyl-biphenyl-4-yl)-5H-imidazo[4,5-c]pyridine; 1-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-propan-2-one; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-prop-2-ynyl-5H-

imidazo[4,5-c]pyridine; 4-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-3-ethoxy-but-2-enoic acid ethyl ester; 5-benzyl-2-(3',4'-dimethyl-biphenyl-4-yl)-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-pyridin-3-ylmethyl-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-pyridin-4-ylmethyl-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-pyridin-2-ylmethyl-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-[1-(toluene-4-sulfonyl)-1H-imidazol-2-ylmethyl]-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-[1-(toluene-4-sulfonyl)-1H-imidazol-4-ylmethyl]-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(5-methyl-[1,3,4]oxadiazol-2-ylmethyl)-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-thiazol-2-ylmethyl-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-[2-(1H-imidazol-4-yl)-ethyl]-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(3-methyl-3H-imidazol-4-ylmethyl)-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(1-methyl-1H-imidazol-4-ylmethyl)-5H-imidazo[4,5c]pyridine; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2-methyl-1H-imidazol-4-yl)-ethanone; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone; 1-(2-Amino-4-methyl-thiazol-5-yl)-2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-ethanone; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-[2-(2H-pyrazol-3-yl)-ethyl]-5H-imidazo[4,5-c]pyridine; 2-[2-(4'-methoxy-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone; 2-{2-[4-(1-methyl-5-trifluoromethyl-1H-pyrazol-3-yl)-phenyl]-imidazo[4,5-c]pyridin-5-yl}-1-(2H-pyrazol-3-yl)-ethanone; 2-[2-(4'-fluoro-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone; 2-[2-(3'-methyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone; 2-[2-(3'-Chloro-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanone; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(2H-pyrazol-3-ylmethyl)-5H-imidazo[4,5-c]pyridine; 2-(3'-chloro-biphenyl-4-yl)-5-(2H-pyrazol-3-ylmethyl)-5H-imidazo[4,5-c]pyridine; 2-(3'-methyl-biphenyl-4-yl)-5-(2H-pyrazol-3-ylmethyl)-5H-imidazo[4,5-c]pyridine; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-ethanol; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-ethylamine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(1H-tetrazol-5-ylmethyl)-5H-imidazo[4,5-c]pyridine1-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-propan-2-ol; 1-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-propan-2-one O-methyl-oxime; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-N,N-dimethyl-acetamide; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-acetamide; 2-[2-(3',4'-dimethyl-biphenyl-4-

yl)-imidazo[4,5-c]pyridin-5-yl]-N-methyl-acetamide; 5-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-1,2-dihydro-pyrazol-3-one; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(1H-imidazol-2-ylmethyl)-5H-imidazo[4,5-c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(1H-imidazol-4-ylmethyl)-5H-imidazo[4,5-c]pyridine; 5-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-2,4-dihydro-[1,2,4]triazol-3-one; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(1H-[1,2,4]triazol-3-ylmethyl)-5H-imidazo[4,5c]pyridine; 5-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-3H-[1,3,4]oxadiazol-2-one; 5-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-ylmethyl]-[1,3,4]oxadiazol-2-ylamine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(2-methyl-1H-imidazol-4-ylmethyl)-5H-imidazo[4,5c]pyridine; 2-(3',4'-dimethyl-biphenyl-4-yl)-5-(1H-pyrazol-4-ylmethyl)-5H-imidazo[4,5-c]pyridine; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-{2-methyl-1-[2-(2-methyl-1H-imidazol-4-yl)-2-oxo-ethyl]-1H-imidazol-4-yl}-ethanone: 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2-methyl-1H-imidazol-4-yl)-ethanol; 2-[2-(3',4'-dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-(2H-pyrazol-3-yl)-ethanol; 2-[2-(3',4'-Dimethyl-biphenyl-4-yl)-imidazo[4,5-c]pyridin-5-yl]-1-pyrazin-2-yl-ethanol; 2-(4-bromo-phenyl)-3H-imidazo[4,5-c]pyridine; 2-[4-(1-methyl-5-trifluoromethyl-1H-pyrazol-3-yl)-phenyl]-3H-imidazo[4,5-c]pyridine hydrochloride; 2-(4'-methoxy-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine; 2-(4'-fluoro-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine; 2-(3'-methyl-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine; and 2-(3'-chloro-biphenyl-4-yl)-3H-imidazo[4,5-c]pyridine.

4. A pharmaceutical composition comprising the compounds of claims 1, 2 or 3, pharmaceutically acceptable addition salts thereof, or substantially enriched enantiomeric forms thereof, and a pharmaceutically acceptable carrier.

5. A composition as defined in claim 4 in unit dosage form.

6. A method for treating the excessive complement activation in a patient comprising administering to said patient, a therapeutically effective amount of a composition of claim 4.

7. A method for treating complement-mediated tissue damage in a patient comprising administering to said patient a therapeutically effective amount of a composition of claim 4.

8. A method for treating diseases characterized by chronic complement activation, comprising administering to a patient a therapeutically effective amount of a composition of claim 4.

9. A method for treating Alzheimer's disease comprising administering to a patient a therapeutically effective amount of a composition of claim 4.

10. A method for treating Huntington's disease comprising administering to a patient a therapeutically effective amount of a composition of claim 4.

11. A method for treating Pick's disease comprising administering to a patient a therapeutically effective amount of a composition of claim 4.

12. A method for treating asthma comprising administering to a patient a therapeutically effective amount of a composition of claim 4.

13. A method for treating hypersensitive pneumonites comprising administering to a patient a therapeutically effective amount of a composition of claim 4.

14. A method for treating anaphylaxis comprising administering to a patient a therapeutically effective amount of a composition of claim 4.

15. A method for treating or preventing a selected from sepsis, adult respiratory distress syndrome, nephrites, graft rejection, myocardial ischemia/reperfusion injury, and intestinal ischemia/reperfusion injury, comprising administering to a patient a therapeutically effective amount of a composition of claim 4.

16. A method for antagonizing the $C3_a$ receptor in a patient by administering an effective amount of a composition of claim 4.

17. A method for antagonizing the $C5_a$ receptor in a patient by administering an effective amount of a composition of claim 4.

ABSTRACT

Aryl substituted imidazo[4,5-c] pyridine compounds are provided. These compounds are useful in ~~pharamecutical~~ pharmaceutical compositions as C3a antagonists for treating a variety of medical conditions associated with the complement cascade. Methods for treating such conditions are also provided.

Figure 1

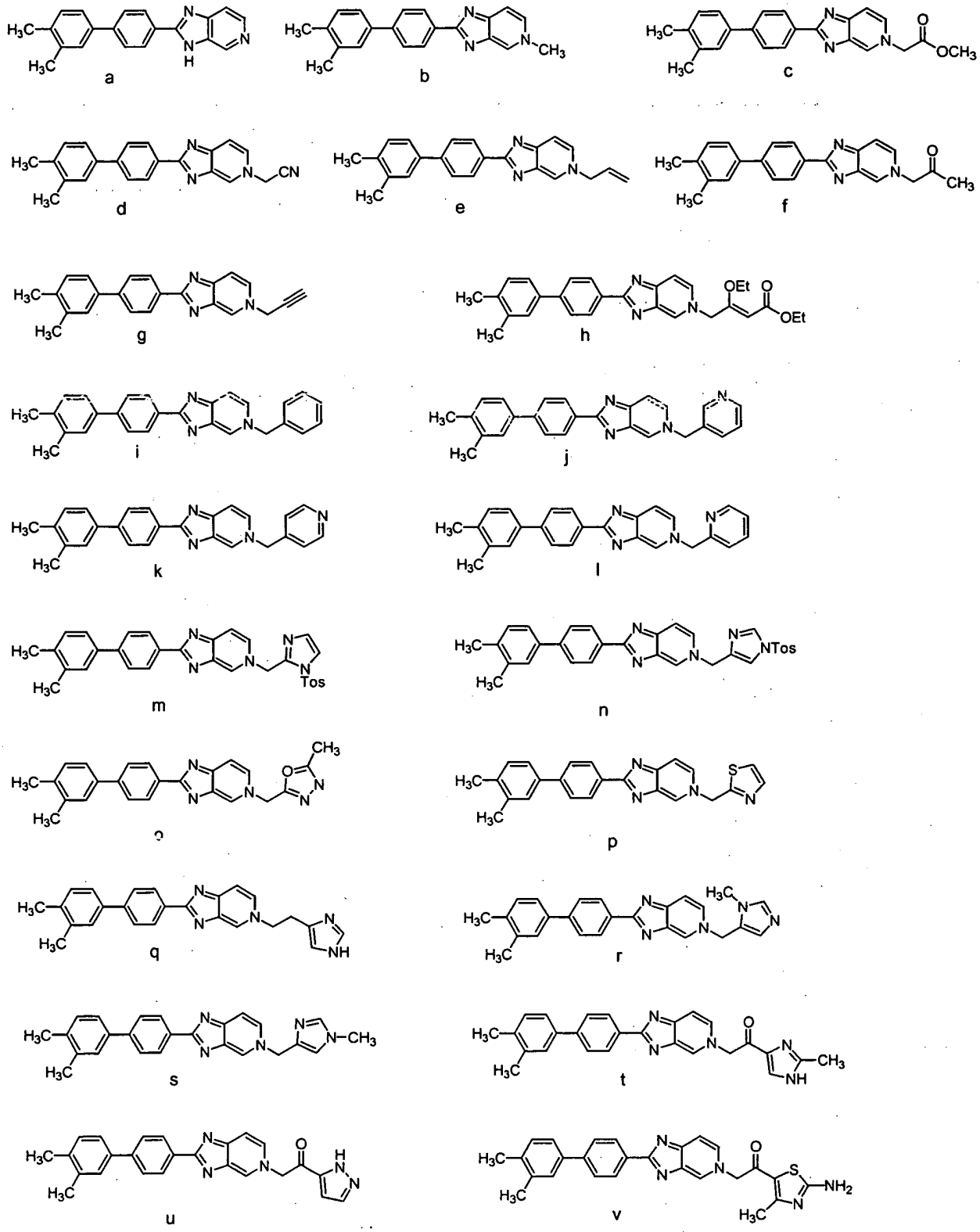


Figure 2

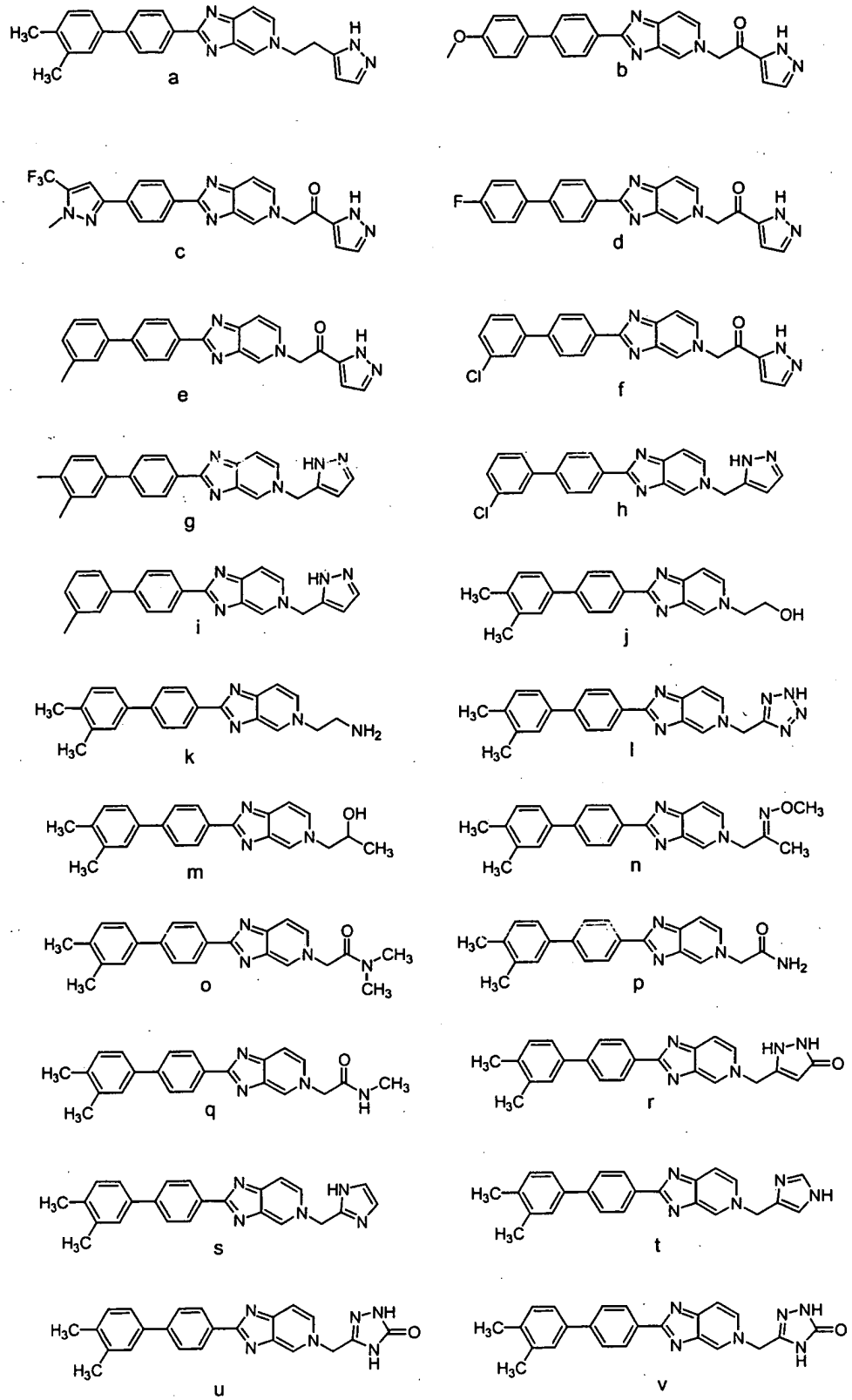


Figure 3

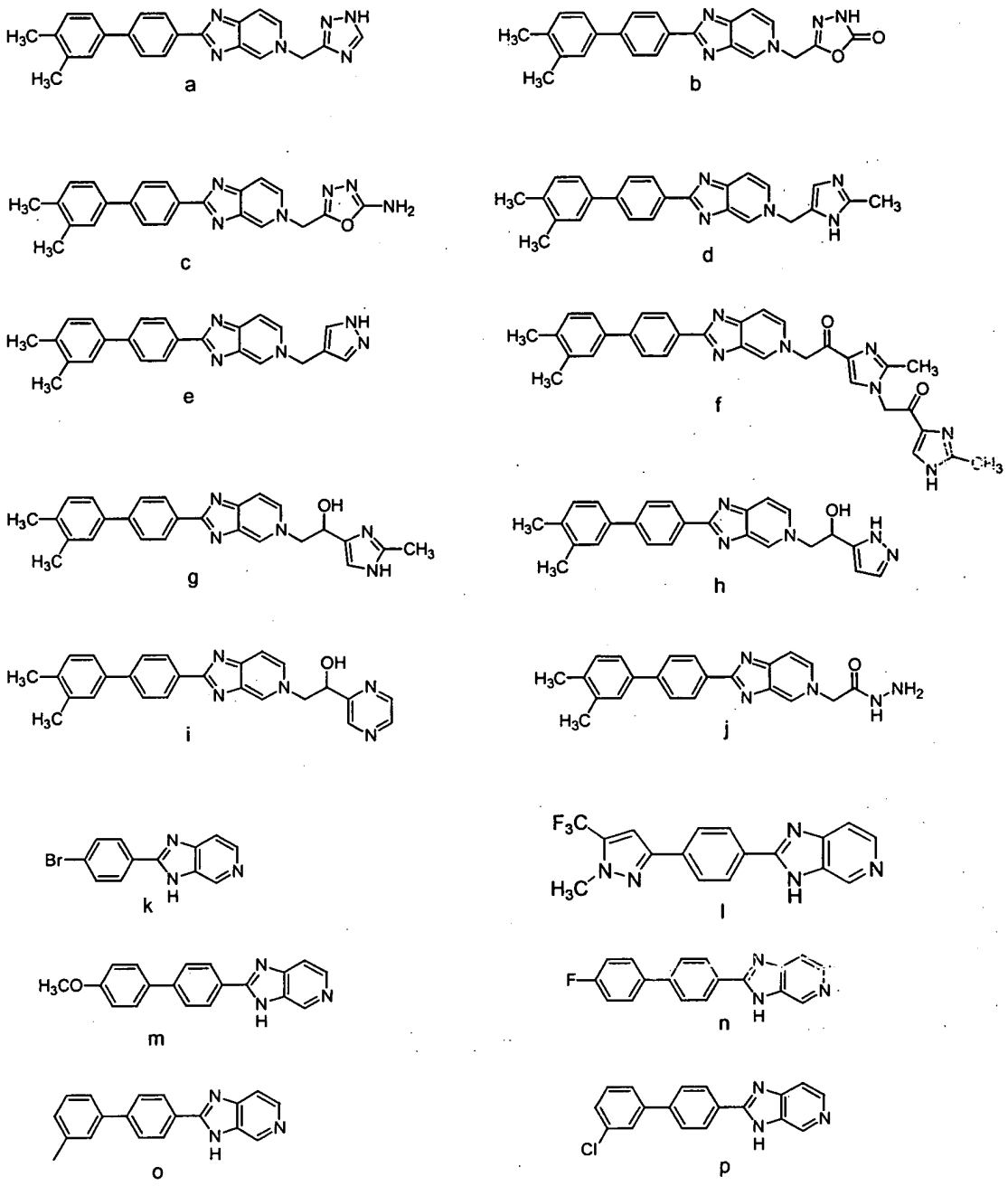
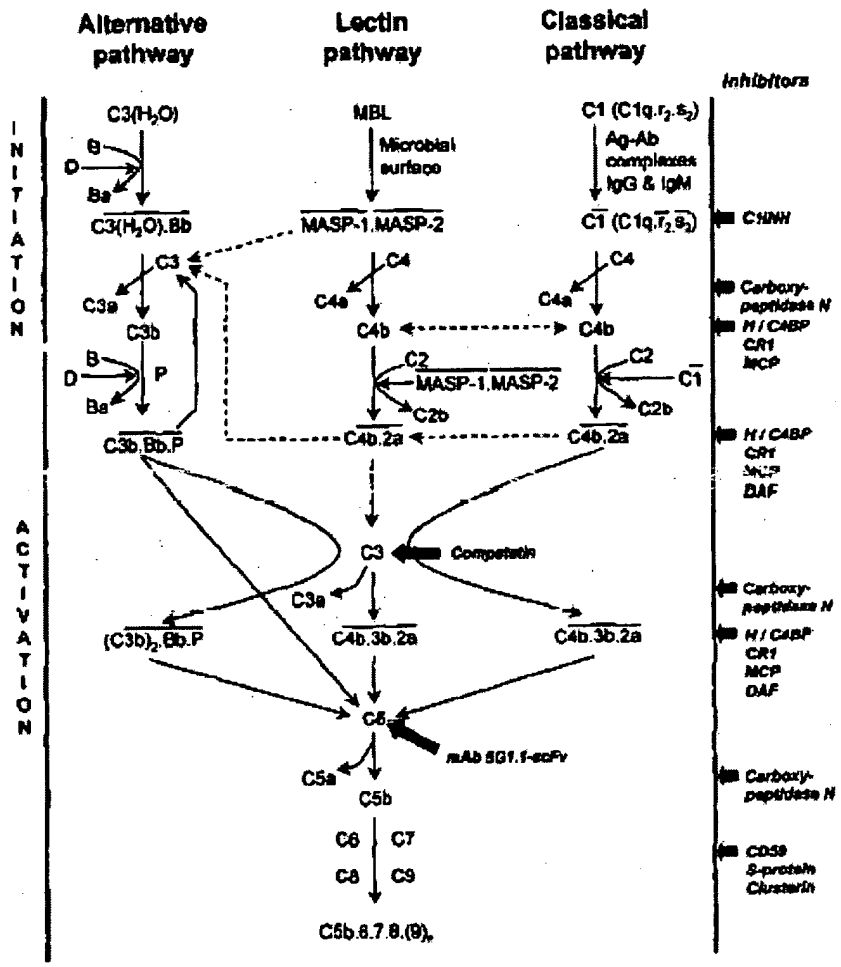


Figure 4



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